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ATTEMPTS AT CULTIVATION OF VIRUSES PRODUCING LEUKOSIS IN FOWLS

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This study was undertaken to investigate the conditions necessary for the multiplication in vitro of tumor-producing viruses. Do these viruses, like most of the viruses [1] that do not produce neoplasms, multiply in the presence of a variety of living cells, or do they depend for multiplication on the presence of certain cells which they endow with neoplastic properties? If a virus multiplies in the presence of normal cells, do these cells acquire the morphologic characteristics of malignant cells, and if by any chance the multiplication of the virus ceases, do they regain their normal appearance?

The problem requires for its solution the use of different viruses and different tissues.

The first problem chosen for investigation was that of the conditions necessary for survival and multiplication in vitro of a virus producing leukosis. In this publication we describe our attempts to cultivate viruses with the ability to produce leukosis by growing cells afflicted with such viruses. In the paper that will follow this one we shall describe further attempts to cultivate viruses producing leukosis in the presence of normal or sarcoma cells and to ascertain whether carcinogenesis can be brought about in vitro.

Several workers have tried to grow a virus producing leukosis in vitro, but without success. Jármai [2] inoculated chick embryos with the virus of leukosis and found that the inoculation was successful only after the ninth day of embryonal life; this is the period when the bone marrow

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1. Tissue culture studies with viruses have been reviewed recently by E. Haagan (Arch. f. exper. Zellforsch. **18**:360, 1936).

2. Jármai, K.: Deutsche tierärtzl. Wchnschr. **41**:418, 1933.

makes its appearance. Jármai concluded that the presence of the cells of bone marrow is necessary for the growth of the virus of leukosis. Furth and Stubbs [3] grew sarcoma cells in vitro in pure culture, which during sixty-seven days of observation elaborated a virus capable of producing both sarcoma and erythroleukosis. Verne, Oberling and Guérin [4] incubated bone marrow of leukotic chickens in coverslip preparations and in Carrel flasks. The blood cells rapidly disappeared, and the cells grown had the characteristics of fibroblasts. An occasional chicken inoculated with a fifteen day culture died of leukemia, while all of those inoculated with older cultures remained healthy. Verne, Oberling and Guérin concluded that the virus of leukosis survives but does not multiply in vitro.

MATERIAL AND METHODS

Cultivation of leukemic blood was attempted in solid and liquid mediums. The solid medium was prepared, according to the well known technic of Carrel, from chicken plasma diluted with Tyrode solution. Heparin was used as an anticoagulant, and embryonic extract as a coagulant and growth-promoting substance. Subpassages were made at approximately weekly intervals. Coverslip preparations were made for histologic studies, according to the usual technic. In the second half of the work ring chambers were used instead of depression slides.[5] The ring chamber has the advantage over the coverslip that the clot is larger and the liquid phase over it keeps it moist.

The liquid cultures were prepared according to the technic of Rous and Beard,[6] in Carrel flasks containing lens paper. Chicken serum was used either undiluted or diluted with an equal amount of Tyrode solution.

The viruses of sarcoma and leukosis used in these studies have been described.[7] Each of these viruses has a characteristic range of action that has remained constant since the first description of the virus. The present study shows that the behavior of these viruses differs among themselves but remains fairly constant and is characteristic for each virus.

Virus 1 produces erythroleukosis and myeloblastic leukemia (Ellermann [8]). All of the European workers studying leukosis have described in recent years the production of sarcoma by their leukotic viruses (Oberling and Guérin,[9] Uhl,

3. Furth, J., and Stubbs, E. L.: Proc. Soc. Exper. Biol. & Med. **32**:381, 1934.

4. Verne, J.; Oberling, C., and Guérin, M.: Compt. rend. Soc. de biol. **121**: 403, 1936.

5. Furth, J., and Breedis, C.: Arch. f. exper. Zellforsch. **18**:246, 1936. Vollmar, H.: Ztschr. f. Zellforsch. u. mikr. Anat. **23**:571, 1935.

6. Rous, P., and Beard, J. W.: J. Exper. Med. **59**:577, 1934.

7. (*a*) Furth, J.: Proc. Soc. Exper. Biol. & Med. **27**:155, 1929; J. Exper. Med. **53**:243, 1931; (*b*) **58**:253, 1933; (*c*) **63**:127, 1936. (*d*) Stubbs, E. L., and Furth, J.: ibid. **61**:593, 1935. (*e*) Furth, J.: ibid. **63**:145, 1936.

8. Ellermann, V.: The Leucosis of Fowls and Leucemia Problems, London, Gyldendal, 1921.

9. Oberling, C., and Guérin, M.: Bull. Assoc. franç. p. l'étude du cancer **22**:180, 1933.

Engelbreth Holm and Rothe-Meyer [10] and Jármai [11]). It is noteworthy that our leukotic virus 1, first described in 1929,[7a] has not produced sarcoma.

Virus 2 [7b] produces lymphomatosis, myelocytomatosis and endothelioma. It does not produce sarcoma after intramuscular injection.

Virus 12 [7c] is derived from virus 2. It produces osteochondrosarcoma and the types of leukosis produced by virus 2.

Virus 13 [7d] produces sarcoma in the breast muscle into which it has been injected, endothelioma of the blood-forming organs and erythroleukosis.

Virus 11 [7e] produces a fibrosarcoma similar to that described by Rous.[12]

CULTURES OF TISSUES FROM A CHICKEN WITH LEUKOSIS PRODUCED BY VIRUS 1

Our first attempts, made in 1934, to cultivate the virus of leukosis in vitro according to the technic of Carrel were unsuccessful, as were those of Verne, Oberling and Guérin.[4]

TABLE 1.—*Inoculations with a Culture of Bone Marrow from a Chicken with Leukosis Produced by Virus 1 (Experiment 1)*

Age of Culture, Days	Passage	Chickens Given Injections	Successful Injections	Length of Life After Injection, Days	
				Successful Injections*	Unsuccessful Injections*
—	Immediate	5	5	D, 24-52 K, 40-43	.....
15	1st and 2d	4	3	D, 47-67	K, 122
25	2d and 3d	4	0	.....	K, 104-106
29	4th	5	0	.....	D, 23- 90 K, 38-100

* K indicates that the chickens were killed; D, that they died.

Experiment 1.—Bone marrow of a chicken with erythroleukosis of strain 1 was cultured in Carrel flasks, using plasma and serum from the same chicken. In the passages, plasma and serum from healthy chickens were used. The results of inoculations with these cultures are shown in table 1. All chickens were given both intramuscular and intravenous injections.

A detailed histologic study of this set of cultures was not made. Round cells disappeared completely in the first few weeks, in accord with observations of previous workers, and after the first passage the culture was composed of fibroblast-like cells only. These cells were slender and fairly uniform in size; the nuclei were likewise fairly uniform in size and shape and contained one or two nucleoli.

Experiment 2.—This experiment, performed two years after the first, was made in a similar manner. The bone marrow of a chicken with myeloid leukemia produced by virus 1 was cultured in Carrel flasks. From time to time ring cultures were set up in order to secure permanent fixed and stained preparations for histologic studies. In the original culture several types of blood cells were seen.

10. Uhl, E.; Engelbreth-Holm, J., and Rothe-Meyer, A.: Acta path. et microbiol. Scandinav. **13**:434, 1936.

11. Jármai, K.: Arch. f. wissensch. u. prakt. Tierh. **69**:275, 1935.

12. Rous, P.: J. Exper. Med. **12**:696, 1910; **13**:397, 1911.

The presence of vast numbers of histiocytes with many epithelioid forms is noteworthy. Mononuclear cells with basophilic cytoplasm were relatively few; polymorphonuclears were abundant.

After the third passage the cultures were composed of cells of two types. Cells of one of these types, with the appearance of fibroblasts are illustrated in figure 1 *A* and *B*. The cells of the second type resembled both myeloblasts of Ellermann and monocytes of the circulating blood (fig. 1 *C* and *D*). The evidence that will be presented supports the view that these cells were identical with the myeloblasts of Ellermann, and we shall refer to them as myeloblasts. There were numerous mitotic figures among both the fibroblasts and the myeloblasts. The myeloblasts were nearer the coverslip than the fibroblasts, and for this reason the photomicrographs do not show both in the same picture. In the course of subsequent passages, the myeloblasts became fewer, but they were found in all of the cultures used for inoculation.

The myeloblasts of Ellermann resemble closely monocytes of the circulating blood but do not exhibit phagocytic properties and do not show rosette formation in vitally stained preparations; in an occasional cell large purple granules, like

TABLE 2.—*Inoculations with a Culture of Bone Marrow from a Chicken with Leukosis Produced by Virus 1 (Experiment 2)*

Age of Culture, Days	Passage	Chickens Given Injections	Successful Injections	Length of Life After Injection, Days	
				Successful Injections*	Unsuccessful Injections*
—	Immediate	5	3	D, 34, 34, 51	K, 86, 98
24	1st	4	1	D, 20	D, 22, 96; K, 98
28	3d	2	0	.....	D, 76; K, 94
38	4th	3	2	D, 57, 66	D, 24
56	5th and 6th	5	3	D, 57, 58, 74	D, 63, 88
62	7th	4	2	D, 50, 51	D, 29, 44

* K indicates that the chickens were killed; D, that they died.

those seen in early myelocytes, are found. These findings support the view of Ellermann that the cells are myeloblasts.

The similar cells in the tissue cultures of experiment 2 gave no evidence of phagocytosis and did not assume microglia-like and epithelioid forms such as were seen in cultures of monocytes,[13] but they had more abundant and less basophilic cytoplasm than the myeloblasts seen in the circulating blood in myeloid leukemia of chickens.

The results of inoculations with these cultures are shown in table 2. All chickens were given intramuscular and intravenous injections.

The results of experiment 1 and the studies of Verne, Oberling and Guérin indicate that the virus of leukosis does not multiply in the presence of fibroblastic cells. It seems probable, therefore, that the multiplication of the virus in the tissue cultures of experiment 2 was due to the presence of the round cells resembling myeloblasts, which multiplied in symbiosis with the fibroblast-like cells. The observation that these cells were round and did not show the various forms assumed by normal

13. Dunning, H. S., and Furth, J.: Am. J. Path. **11**:895, 1935.

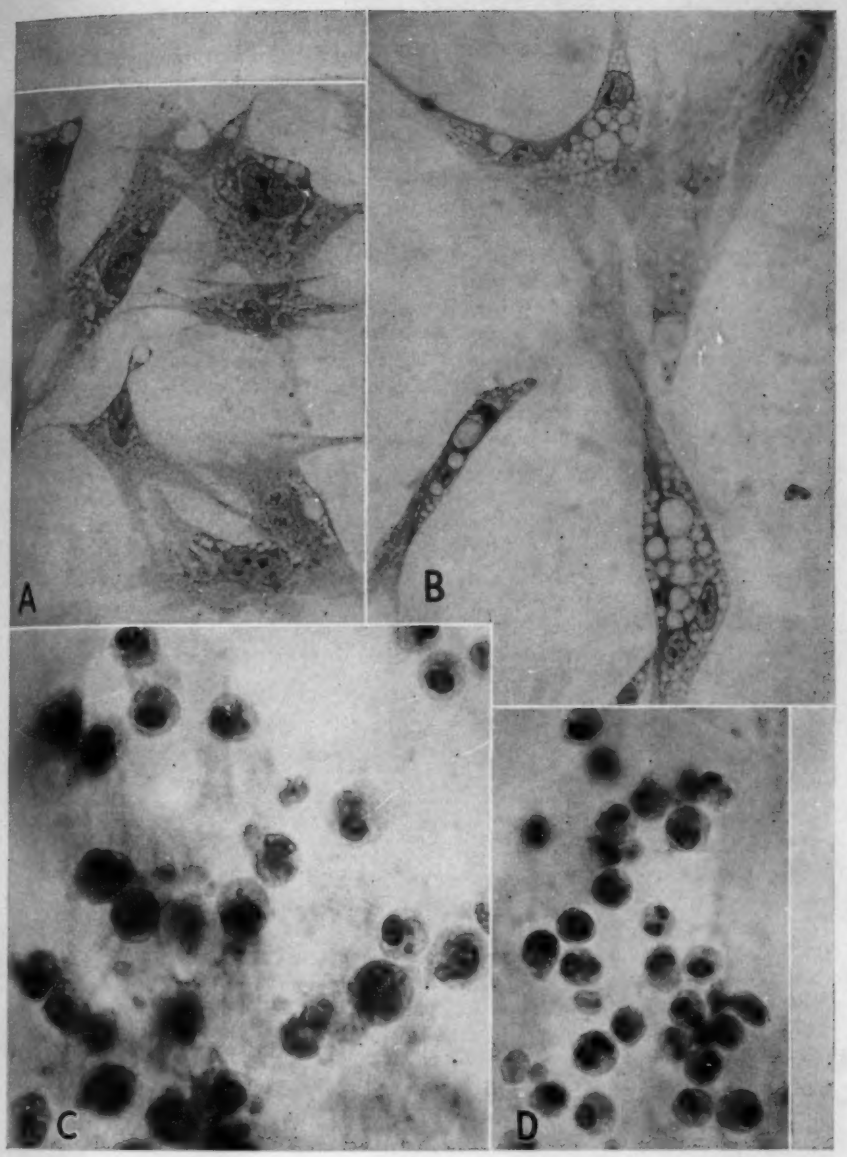

Fig. 1.—Figures 1 to 6 present tissue cultures which were fixed in formaldehyde and ammonium bromide and stained with hematoxylin. The blood smears were stained with Wright and Giemsa solutions. The magnifications stated are only approximate.

In figure 1, *A* shows a three day growth of fibroblastic cells from the third passage of a culture of bone marrow from a chicken with myeloid leukemia (experiment 2); × 500. *B* shows the same culture five days after incubation; × 500. *C* presents mononuclear cells, presumably myeloblasts, in the same culture; × 800. *D* presents cells of the same type as in *C*; ×600.

The presence of vast numbers of histiocytes with many epithelioid forms is noteworthy. Mononuclear cells with basophilic cytoplasm were relatively few; polymorphonuclears were abundant.

After the third passage the cultures were composed of cells of two types. Cells of one of these types, with the appearance of fibroblasts are illustrated in figure 1 *A* and *B*. The cells of the second type resembled both myeloblasts of Ellermann and monocytes of the circulating blood (fig. 1 *C* and *D*). The evidence that will be presented supports the view that these cells were identical with the myeloblasts of Ellermann, and we shall refer to them as myeloblasts. There were numerous mitotic figures among both the fibroblasts and the myeloblasts. The myeloblasts were nearer the coverslip than the fibroblasts, and for this reason the photomicrographs do not show both in the same picture. In the course of subsequent passages, the myeloblasts became fewer, but they were found in all of the cultures used for inoculation.

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				Successful Injections*	Unsuccessful Injections*
—	Immediate	5	3	D, 34, 34, 51	K, 86, 98
24	1st	4	1	D, 20	D, 22, 96; K, 98
28	3d	2	0	.....	D, 76; K, 94
38	4th	3	2	D, 57, 66	D, 24
56	5th and 6th	5	3	D, 57, 58, 74	D, 63, 88
62	7th	4	2	D, 50, 51	D, 29, 44

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The similar cells in the tissue cultures of experiment 2 gave no evidence of phagocytosis and did not assume microglia-like and epithelioid forms such as were seen in cultures of monocytes,[13] but they had more abundant and less basophilic cytoplasm than the myeloblasts seen in the circulating blood in myeloid leukemia of chickens.

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13. Dunning, H. S., and Furth, J.: Am. J. Path. **11**:895, 1935.

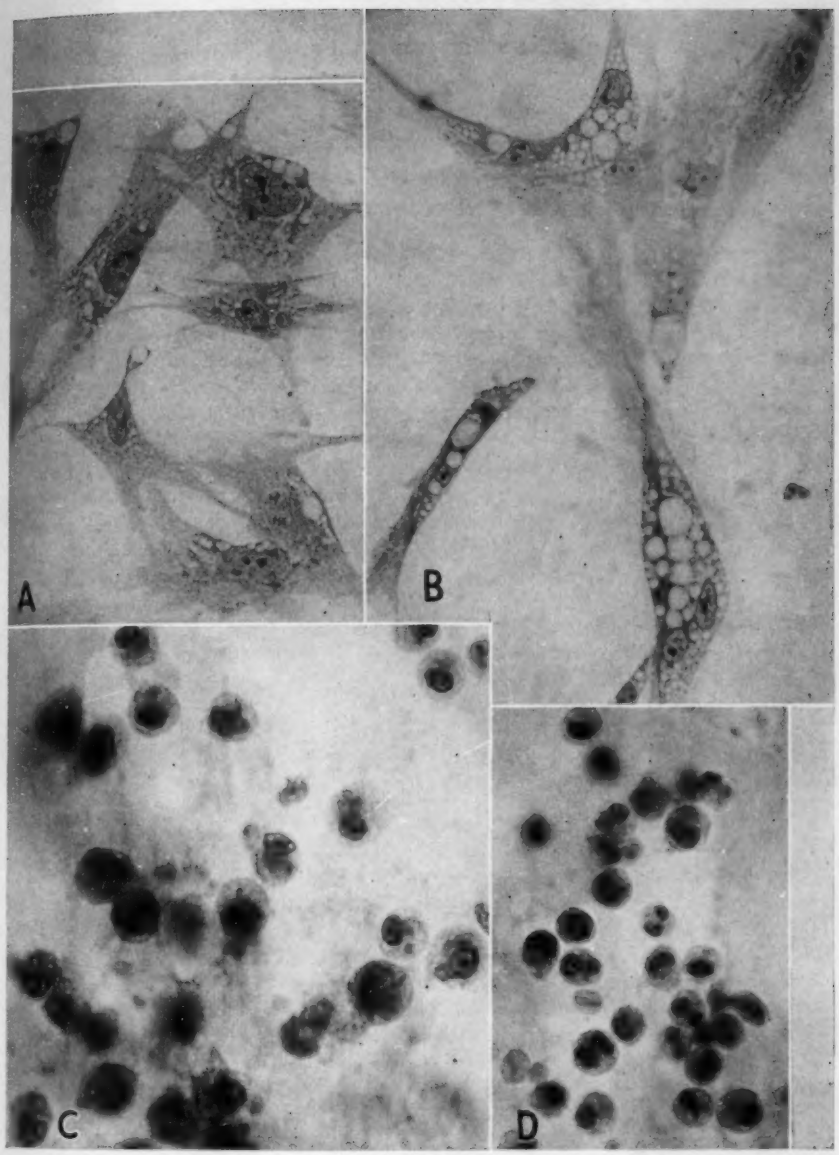

Fig. 1.—Figures 1 to 6 present tissue cultures which were fixed in formaldehyde and ammonium bromide and stained with hematoxylin. The blood smears were stained with Wright and Giemsa solutions. The magnifications stated are only approximate.

In figure 1, *A* shows a three day growth of fibroblastic cells from the third passage of a culture of bone marrow from a chicken with myeloid leukemia (experiment 2); × 500. *B* shows the same culture five days after incubation; × 500. *C* presents mononuclear cells, presumably myeloblasts, in the same culture; × 800. *D* presents cells of the same type as in *C*; ×600.

monocytes in vitro lends further support to the view that they were myeloblasts. Moreover, in solid cultures of normal bone marrow and spleen, monocytes were present only in the first few passages.

A similar symbiotic culture of fibroblasts with myeloblasts was obtained by incubating leukotic spleen (experiment 3), and inoculations with these cultures likewise produced leukosis.

Experiment 3.—Cultures were set up from the spleen of the chicken with myeloblastic leukemia the marrow of which was cultured in experiment 2. In figure 2, *A* and *B* show the margin of the explant after twenty-four hours of incubation, and here the predominant cells resembled myeloblasts of circulating blood. Several mitotic forms were seen among these cells. In *A*, the arrow points to a monocyte. The monocytes in contrast to the myeloblasts were pale staining and had smaller nuclei and abundant cytoplasm. Many of them had ingested erythrocytes or cell fragments, while the myeloblasts showed no phagocytic activity. Phagocytic mononuclear cells were seen only in the original culture.

TABLE 3.—*Inoculations with Cultures of Spleen from a Chicken with Leukosis Produced by Virus 1 (Experiment 3)*

Age of Culture, Days	Passage	Chickens Given Injections	Successful Injections	Length of Life After Injection, Days	
				Successful Injections*	Unsuccessful Injections*
—	Immediate	5	2	D, 29, 48	K, 65, 95, 95
24	2d	2	1	D, 52	D, 17
28	3d	3	2	D, 48; K, 58	K, 94
56	4th-7th	7	3	D, 37, 41, 44	D, 22, 41, 48; K, 48
62	8th	6	0	.....	D, 19, 36, 49, 78; K, 90, 90

* K indicates that the chickens were killed; D, that they died.

In the course of successive passages the myeloblast-like cells became less abundant, and the cultures were composed almost entirely of fibroblast-like cells (fig. 2 *C* and *D*). The presence of myeloblasts, however, was established even in the seventh passage. They resembled closely the round cells seen in the cultures of bone marrow in experiment 2 and illustrated in figure 1 *C* and *D*.

The results of the inoculations are shown in table 3. All chickens were given intramuscular and intravenous injections. In the cultures of the eighth passage, no mononuclear cells were seen, and inoculations of these cultures yielded no leukosis, whereas all of the earlier cultures contained myeloblast-like cells and produced leukosis.

Experiments 1, 2 and 3 show that it is possible to keep alive in vitro the agent transmitting leukosis for at least sixty-two days. All of the cultures which produced leukosis contained cells like the myeloblasts of Ellermann in addition to fibroblast-like cells.

Experiment 4: Cultures of Leukemic Blood Cells in Liquid Mediums.—Blood of a chicken with leukosis produced by virus 1, showing numerous erythroblasts and a few myeloblasts, was drawn into a chilled syringe containing a few drops of a 1:100 solution of heparin. The blood was centrifugated at 800 revolutions per minute for six minutes. The white cell layer containing a considerable number of red cells was placed on lens paper in Carrel flasks, as described by Rous and

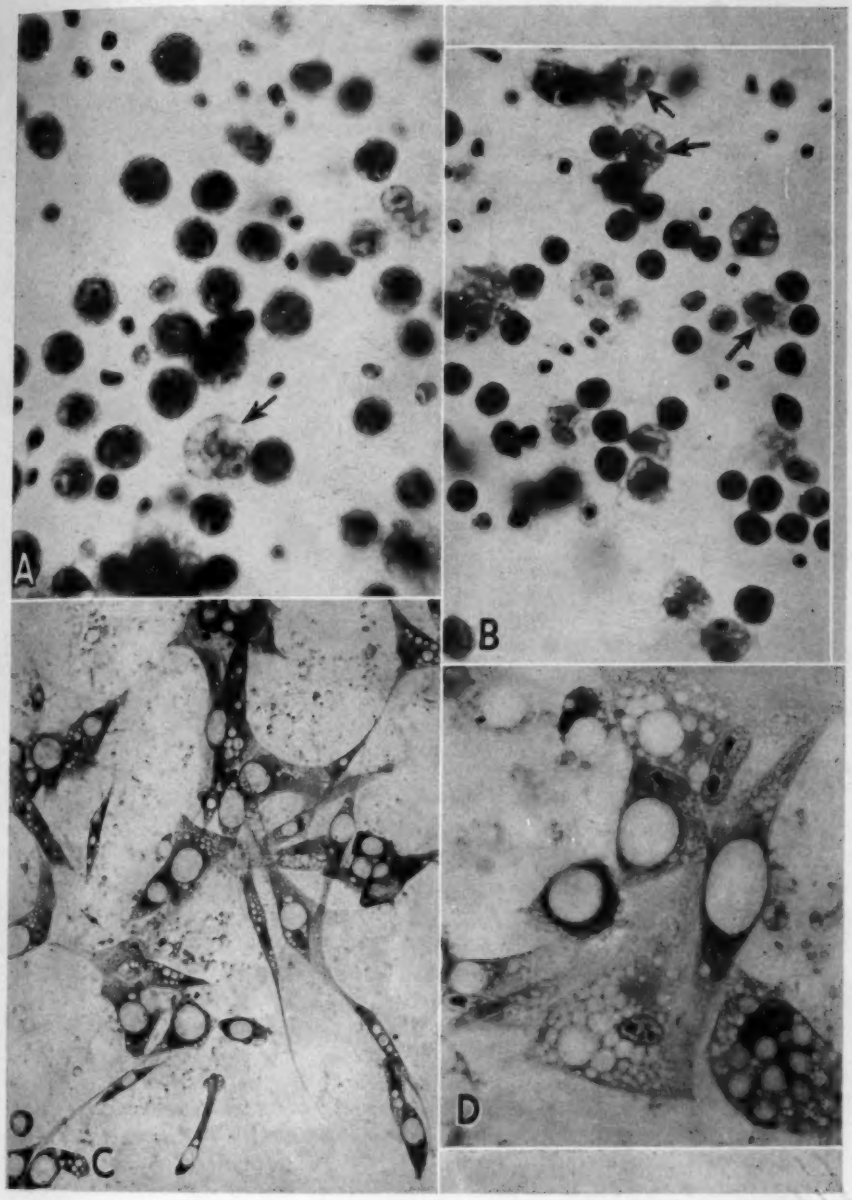

Fig. 2.—*A* shows 1 day old myeloblasts with few monocytes (one indicated by arrow) in a one day culture of spleen from a leukotic chicken (experiment 3); × 800. *B* shows the same cells; × 700. *C* shows fibroblasts in the seven day culture of the same leukotic spleen; × 250. *D* shows the same cells as *C*; × 500.

Beard.[6] Undiluted serum of the leukotic chicken was used as the nutrient liquid. On each succeeding day the serum in the flasks was pipetted off and replaced by serum of normal chickens. After seven days, about half of the cells were removed from one flask and transferred to a new flask containing lens paper and normal serum. To obtain material for inoculation the flasks were shaken to free the cells on the lens paper, and about one half of the cell suspension was removed. The samples removed during the first three weeks of incubation were centrifugated at about 3,000 revolutions per minute for ten minutes and the supernatant fluid and sediment were injected into different chickens. The results of the inoculations with these cultures are shown in table 4. All chickens were given intravenous injections.

TABLE 4.—*Inoculations with Cultures of Myeloblasts from a Chicken with Leukosis Produced by Virus 1 (Experiment 4)*

Age of Culture, Days	Passage	Material Injected	Chickens Given Injections	Successful Injections	Length of Life After Injection, Days: Successful Injections*	Length of Life After Injection, Days: Unsuccessful Injections*
Immediate	Original	Cells	2	2	K, 30; D, 30	.....
	Original	Supernatant fluid	2	1	D, 84	K, 95
8	Original	Cells	2	2	D, 37, 44	.....
	Original	Supernatant fluid	2	0	.....	K, 88, 88
13	Original	Cells	1	1	D, 39	.....
	Original	Supernatant fluid	1	0	.....	K, 82
16	Original	Cells	1	1	D, 35	.....
	Original	Supernatant fluid	1	0	.....	K, 99
28	Original	Cells and supernatant fluid	1	0	.....	K, 102
	1st	Cells and supernatant fluid	1	0	.....	K, 102
30	Original	Cells and supernatant fluid	1	0	.....	K, 100
	1st	Cells and supernatant fluid	2	1	K, 64	K, 23
32	Original	Cells and supernatant fluid	1	0	.....	K, 98
	1st	Cells and supernatant fluid	2	1	K, 38	K, 98

* K indicates that the chickens were killed; D, that they died.

Table 4 shows that the virus of leukosis produced by virus 1 can be kept alive for at least thirty-two days by incubating the leukemic blood in serum according to the technic of Rous and Beard.

Smears were prepared from time to time to ascertain the type of cells present in the cultures. Smears of the original culture after four and nine days of incubation contained vast numbers of myeloblasts, several in mitoic division (fig. 3*A* to *E*). The cells examined were not phagocytic. In the smears taken at later intervals, smudged cells appeared in increasing numbers, but it was uncertain whether the cells disintegrated in the culture or were crushed in the process of preparation. Cells like myeloblasts persisted until the final injection. Promyelocytic granules were seen in smears up to four days. Mitotic figures were present throughout the life of the culture (fig. 3*F*). Most of the mononuclear cells became vacuolated but still divided by mitosis. The cytoplasm of the cells gradually lost some of its basophilia, and the cells then resembled both monocytes and myeloblasts.

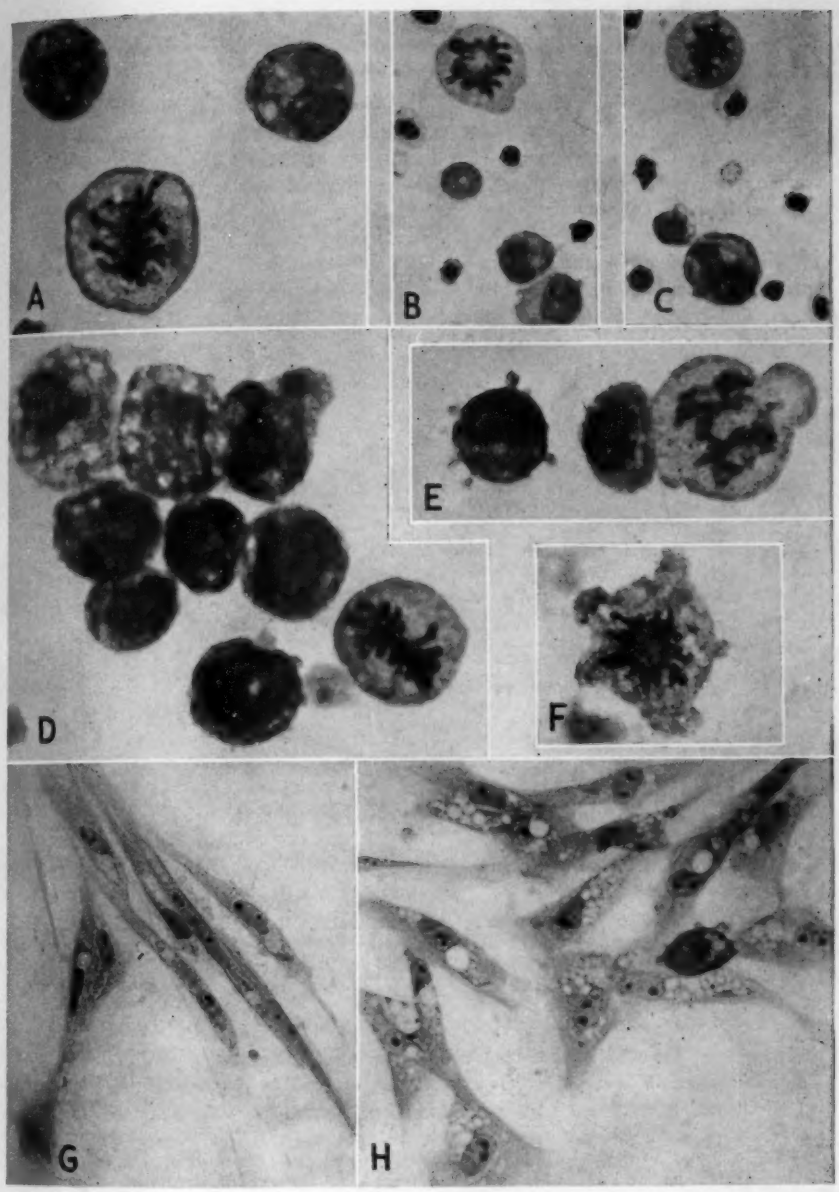

Fig. 3.—*A* to *F* show myeloblasts in smears from the liquid cultures of the blood of a chicken with myeloblastic leukemia (experiment 4). *A* (× 1,000), *B* and *C* (× 800) are from the four day cultures, *D* and *E* from the nine day cultures (× 1,000), and *F* from the thirty day culture (× 1,000). *G* shows fibroblast-like cells in a four day culture of the spleen of a normal chicken in the presence of virus 13 (table 11); × 250. *H* shows a six day growth of the second passage of sarcoma produced by virus 11 grown with normal spleen (table 13); × 400.

Similar results were obtained in a second experiment, which was set up before the results of the first experiment were known.

Experiment 5.—The buffy coat of the blood of a chicken with myeloid leukemia was cultivated on lens paper according to the technic of Rous and Beard, as in the preceding experiment. The serum was replaced daily with fresh normal serum until the seventeenth day, when a mixture of one part of serum and one part of Tyrode solution was used instead. One subpassage was made, as in the previous experiment. All injections were made by the intravenous route (table 5).

In both series of liquid cultures myeloblasts were alive in vitro during the entire period of observation, twenty-six days and thirty-two days, respectively. They multiplied by mitotic division, but multiplication was scant, and more cells died

TABLE 5.—*Inoculations with Cultures of Myeloblasts from a Chicken with Leukosis Produced by Virus 1 (Experiment 5)*

Age of Culture, Days	Passage	Material Injected	Chickens Given Injections	Successful Injections	Length of Life After Injection, Days	
					Successful Injections*	Unsuccessful Injections*
Immediate	.....	White cell layer	4	4	D, 17, 27 K, 17, 22	.....
12	Original	Cells.....	1	1	K, 26	.....
		Supernatant fluid	1	0	.....	K, 97
12	1st	Cells.....	1	0	.....	K, 97
		Supernatant fluid	1	0	.....	K, 97
22	Original	Cells and supernatant fluid....	1	0	.....	K, 102
	1st	Cells and supernatant fluid....	1	1	D, 35	.....
24	Original	Cells and supernatant fluid....	1	0	.....	K, 100
	1st	Cells and supernatant fluid....	1	1	K, 50	.....
26	Original	Cells and supernatant fluid....	1	0	.....	K, 98
	1st	Cells and supernatant fluid....	1	1†	K, 100	.....

* K indicates that the chickens were killed; D, that they died.
† This chicken had chronic myeloid leukemia, from which it recovered; at autopsy neurolymphomatosis was found.

than were produced by mitotic division. The cells certainly did not thrive in the cultures, as indicated by the extensive vacuolation of their cytoplasm. With increasing age of the culture in experiment 5 there was conspicuous lengthening of the duration of illness, although successful inoculations were obtained up to the last day of observation.

Summary of Results with Virus 1.—In solid cultures of bone marrow and spleen of a chicken with myeloblastic leukemia (experiments 2 and 3), mononuclear cells, presumably myeloblasts, proliferated in symbiosis with fibroblast-like cells, and these cultures, when inoculated into chickens, produced leukosis. Incubation of bone marrow of a chicken with erythroleukosis (experiment 1) yielded fibroblastic growth only, and these cultures did not produce leukosis.

Leukemic myeloblasts when incubated in vitro in liquid culture (experiments 4 and 5) undergo mitotic division for at least thirty-two days and retain the ability to produce leukosis.

CULTURES OF SARCOMA CELLS FROM A CHICKEN INFECTED WITH THE COMPLEX (SARCOMA-LEUKOSIS) VIRUS 13

In recent years numerous workers (Oberling and Guérin;[9] Uhl, Rothe-Meyer and Engelbreth Holm;[10] Jármai[11]) have described viruses that produce both sarcoma and leukosis. One of us (J. Furth), in association with Dr. Stubbs,[7d] has studied a similar virus, named virus 13, and arrived at the conclusion that it has affinities for primitive blood cells, endothelial cells and probably also for common fibroblasts. If this opinion is correct, a pure culture of sarcoma cells produced by this virus should elaborate a virus capable of producing leukosis.

Two series of tissue cultures were made, with sarcomas produced by virus 13, and the results of inoculations with these cultures are shown in tables 6 and 7. Experiment 6 (table 6) has been briefly described in a preliminary report.[3]

Experiments 6 and 7.—The sarcoma produced in the breast muscle by virus 13 was cultured in a plasma clot according to the technic of Carrel. The culture was set up in the donor's plasma, and its serum was used as nutrient. The subpassages were made in normal plasma and serum. Particles of normal spleen were added to the supernatant fluid of the sixty-four day cultures immediately before injection.

After the first subpassage there were, in the culture, no cells other than those resembling fibroblasts. The morphologic appearance of the cells in cultures of the seventh passage of experiment 7 is illustrated in figure 4 *A* to *D*. They were of the size of fibroblasts, elongated or polygonal, with few processes. The nuclei were large and oval, and each contained from 1 to 4 small nucleoli. Mitotic figures were abundant. The mitotic form was almost invariably round, and the chromosomes formed a small compact mass. A small number of multinucleated giant cells were seen. In the central part of almost every colony was a liquefied area invaded by fibroblast-like cells growing on the glass. There was great variation in size and shape in the cells and their nuclei. In older cultures a great deal of fat was found in the cytoplasm of the cells. Some of the cells were very small, but the presence of continuous transitional forms between the large and the small cells indicates that the cultures were composed of cells of a single type.

Inoculations with a pure culture of sarcoma cells produced both sarcoma and leukosis after sixty-seven days of incubation in experiment 6 and after one hundred and fifty-eight days of incubation in experiment 7. When the cultures lost the ability to produce sarcoma they lost the ability to produce leukosis. The failure to maintain the virus after sixty-seven and one hundred and fifty-eight days, respectively, was associated with difficulties in making further passages of the cultures. The colonies formed were small, and the cells degenerated after a few days. In experiment 7 the amount of tissue injected at the end of the experiment (two hundred and thirty-four days) was doubtless smaller than that injected on the preceding days.

Summary of Results with Virus 13.—The results of experiments 6 and 7 support the view that a single virus may produce both sarcoma

TABLE 6.—*Inoculations with Cultures of Sarcoma Cells from a Chicken with Sarcoma Produced by Virus 13 (Experiment 6)*

Age of Culture, Days	Passage	Material Injected	Route of Injection*	Chickens Given Injections	Successful Injections†	Length of Life After Injection, Days: Successful Injections‡	Length of Life After Injection, Days: Unsuccessful Injections‡
12	Original	Supernatant fluid and culture particles	I.v. I.m.	2	1 S 1 SE	K, 77 K, 28	....
	1st	Supernatant fluid and culture particles	I.v. I.m.	2	1 S 1 SE	D, 25 D, 25	....
23	3d	Culture particles	I.m.	5	2 S 2 SE	K, 36; D, 36 K, 28; D, 37	..... K, 105§
35	3d, 4th	Supernatant fluid and culture particles	I.v. I.m.	4	2 S 1 S(E?) 1 SE	D, 34 K, 25 D, 34	....
67	8th, 9th, 10th	Supernatant fluid	I.v.	3	1 S 1 SE	D, 47 D, 42	..... K, 97
		Particles	I.m.	3	2 S 1 SE	K, 17, 56 D, 37	....
102	16th, 17th	Supernatant fluid and culture particles	I.v. I.m.	4	0	.....	K, 87, 87, 87, 87
130	17th, 18th	Supernatant fluid and culture particles	I.v. I.m.	3	0	.....	K, 46,# 103, 103
133	17th, 18th	Supernatant fluid and culture particles	I.v. I.m.	3	0	.....	K, 75, 103, 143

* I.v. indicates intravenous; I.m., intramuscular.
† S signifies sarcoma; E, erythroleukosis.
‡ K indicates that the chickens were killed; D, that they died.
§ This chicken had lymphomatosis.
#This chicken had neurolymphomatosis.

TABLE 7.—*Inoculation with Cultures of Sarcoma Cells from a Chicken with Sarcoma Produced by Virus 13 (Experiment 7)*

Age of Culture, Days	Passage	Material Injected	Route of Injection*	Chickens Given Injections	Successful Injections†	Length of Life After Injection, Days: Successful Injections‡	Length of Life After Injection, Days: Unsuccessful Injections‡
Immediate	Original	Supernatant fluid	I.v.	4	1 SE 1 (S?)	D, 40 K, 98	K, 86, 149 .....
		Culture particles	I.m.	4	3 SE 1 S	K, 38, 46, 47 D, 128	....
14	Original, 2d	Culture particles	I.m.	4	2 S 1 SE 1 S(E?)	K, 63, 79 K, 44 K, 39	....
47	1st to 6th	Supernatant fluid	I.v.	3	0	.....	K, 101, 101; D, 79
		Culture particles	I.m.	3	2 SE 1 SM	D, 37, 42 K, 47	....
64	2d to 6th	Culture particles	I.m.	5	2 S 2 SE 1 SM	K, 39, 66 K, 36, 51 D, 65	....
84	1st to 7th	Supernatant fluid Culture particles	I.m. I.m.	3 4	0 1 SE	..... K, 45	K, 52, 58,§ 115 D, 21; K, 96,§ 115
122	5th to 7th	Culture particles	I.m.	2	2 SE	D, 35; K, 40	.....
158	6th to 9th	Supernatant fluid and culture particles	I.v. I.m.	4	2 S 1 SE	K, 36; D, 57 D, 38	K, 78 .....
224	9th to 11th	Supernatant fluid and culture particles	I.v. I.m.	3	1 S	D, 63	K, 98, 98
235	11th to 12th	Supernatant fluid and culture particles	I.v.	3	0	.....	K, 89, 92, 95

* I.v. indicates intravenous; I.m., intramuscular.
† S signifies sarcoma; E, erythroleukosis; M, myeloid leukemia
‡ K indicates that the chickens were killed; D, that they died.
§ These chickens had neurolymphomatosis.

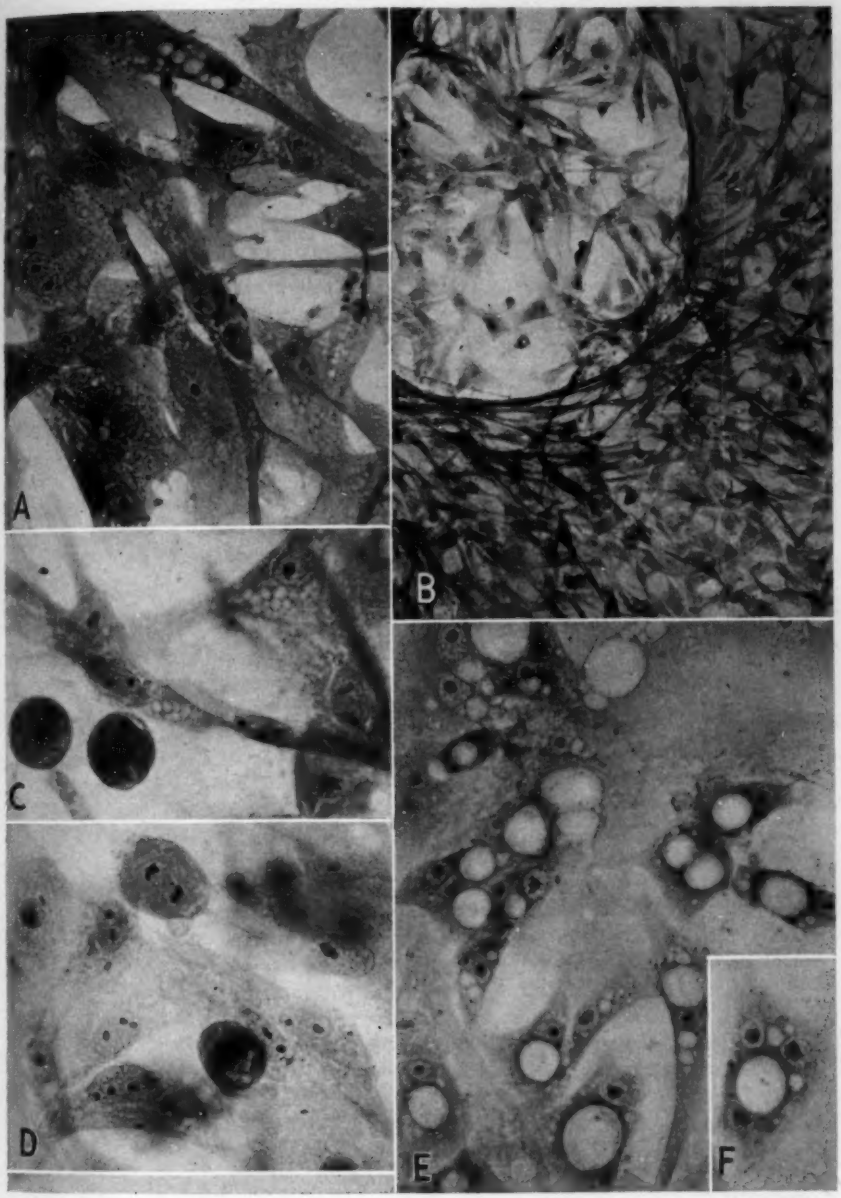

Fig. 4.—*A* to *D* represent two day cultures of the seventh passage of sarcoma produced by virus 13 (experiment 7), under the following magnifications: *A*, × 500; *B*, × 150; *C* and *D*, × 500. *E* and *F* represent sixteen day growths of the second passage of a culture of spleen from a chicken having leukosis produced by virus 2 (experiment 8); × 500.

and leukosis. They show that it is possible to keep this complex virus producing leukosis alive for at least one hundred and fifty-eight days in cultures of the sarcoma.

CULTURES OF A LEUKOTIC SPLEEN AND OF ENDOTHELIOMA PRODUCED BY VIRUS 2

Two experiments were made with virus 2. In one experiment (8) the spleen of a leukotic chicken, and in the other experiment (9) an endothelial tumor, was cultured.

The culture of the leukotic spleen (experiment 8) yielded fibroblast-like and polygonal cells with pale nuclei and bulky, intensely staining nucleoli, illustrated in figure 4 *E* and *F*. These cells resembled closely the endothelial cells characteristic of this strain and described and illustrated in a previous report.[14] The appearance of the cells remained unaltered during the period of observation, but the

TABLE 8.—*Inoculations with Cultures of Spleen from a Chicken with Leukosis Produced by Virus 2 (Experiment 8)*

Age of Culture, Days	Passage	Chickens Given Injections	Successful Injections	Length of Life After Injection, Days	
				Successful Injections*	Unsuccessful Injections*
7	Original	5	3	K, 43; D, 56, 163	K, 92, 98
27	1st and 2d	3	2	K, 67; D, 93	K, 114
	2d and 4th	2	1	D, 63	K, 114
36	1st to 5th	5	1	D, 45	K, 118, 118, 118, 118

* K indicates that the chickens were killed; D, that they died.

growth became gradually poorer. The cells showed a tendency to migrate into the clot and failed to form compact colonies like those of common fibroblasts.

Inoculations with this culture gave evidence of the presence of the virus until the last day of incubation (thirty-six days). The small percentage of successful injections may be explained by the poor growth of the cells in vitro.

The supernatant fluid of the original culture was injected intravenously and the culture particles intramuscularly; the later injections were intramuscular, and the culture particles were suspended in the supernatant fluid. Virus 2, unlike virus 1, is transmitted just as readily by intramuscular as by intravenous inoculations.

The second culture with virus 2 was prepared from a tumor showing neoplastic endothelial cells and hemocytoblasts. The growth obtained was composed of cells similar to those of experiment 8, characterized by pale staining nuclei with bulky dark staining nucleoli. The cells grown in vitro were similar to those of the endotheliomas produced by virus 2.[14] They could be identified in the tissue cultures during the sixty-four days of cultivation.

In the cultures of the first passages the malignant cells anastomosed one with another, as shown in figure 5 *A*, and few round forms were seen. In the third passage the cells grew freely (fig. 5 *B*), anastomoses were few, and many cells were isolated (fig. 5 *C* and *D*). With less compact growth, transfer becomes more difficult. The colonies were very small, and only a small amount of material was available for inoculation.

14. Furth, J.: J. Exper. Med. **59**:501, 1934.

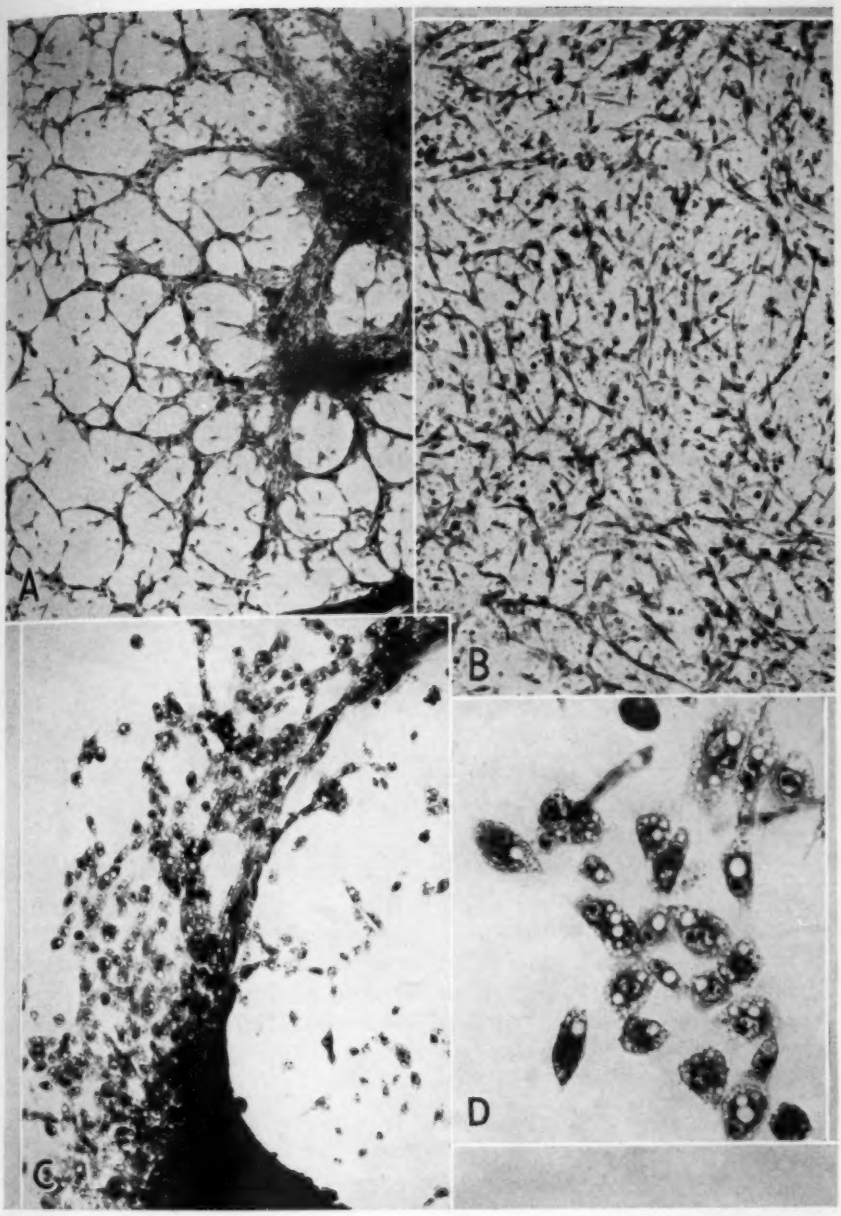

Fig. 5.—*A* represents a six day culture of the first passage of cultures of endothelioma produced by virus 2 (experiment 9); × 100. It shows an anastomosing network of spindle-shaped cells. *B* shows an eight day growth of the third passage of the same culture. It reveals an irregular growth of spindle-shaped cells; × 100. *C* and *D* represent a six day old growth of another ring culture of the same series and show detached polygonal cells, each with a bulky nucleolus. The magnifications are: *C*, × 150; *D*, × 500.

It is noteworthy that in all of four chickens that received injections of cultures after thirty-four days of incubation leukosis developed (table 9). Failure to produce the disease after sixty-four days of incubation may be due either to loss of virulence of the virus or, more likely, to the small amount of material introduced.

Summary of Results with Virus 2.—The leukosis-producing virus 2 could be maintained in vitro during thirty-six days by cultivating leukotic spleen and during thirty-four days by cultivating the neoplastic endothelial cells produced by this virus. The difficulty of maintaining this virus in vitro was associated with difficulties in growing the cells.

CULTIVATION OF TUMORS PRODUCED BY VIRUS 12

Virus 12 produces osteochondrosarcoma and lymphomatosis. It is intimately related to the leukosis virus 2; it took its origin in a chicken inoculated with virus 2 and in the course of five years we have observed

TABLE 9.—*Inoculations with Cultures of an Endothelial Tumor Produced by Virus 2 (Experiment 9)*

Age of Culture, Days	Passage	Chickens Given Injections*	Successful Injections	Length of Life After Injection, Days	
				Successful Injections†	Unsuccessful Injections†
Immediate	Original	3	2	K, 30, 31	D, 27
34	2d to 4th	4	4	K, 56, 62, 88, 98	.....
64	3d to 5th	4	0	.....	K, 34, 93, 100, 104

* The culture particles were injected intramuscularly. The "immediate" injections were made with cut-up tumor particles.
† K indicates that the chickens were killed; D, that they died.

the development of bone tumors in three of approximately twelve hundred chickens inoculated with virus 2. No bone tumors were observed in a greater number of birds used in the studies of viruses other than nos. 2 and 12. The relation of virus 2 to virus 12 has not been definitely established. Virus 2, unlike virus 12, has not produced osteochondrosarcoma, with the possible exception of the three cases mentioned, when injected through the sternal keel or about the periosteum of other bones. In approximately one third of the chickens inoculated with virus 12 leukosis developed, unassociated with sarcoma. The chickens inoculated with tissues deriving from these leukotic chickens did not produce bone tumor, whereas transmissions from chickens with osteochondrosarcoma yielded both osteochondrosarcoma and leukosis (Furth [7c]; also, unpublished data). These findings resemble the well known dissociation phenomena of micro-organisms. The tissue culture studies with virus 12 were undertaken to obtain further information on the relation of leukosis to bone sarcoma.

Osteogenesis in normal tissues of an adult animal in vitro has thus far not been observed. We hoped to obtain a culture of neoplastic osteo-

blasts in order to determine whether bone formation occurred in it. Four series of cultures were made, but osteogenesis was not observed.

In experiments 10, 11 and 13, the initial cultures were prepared from an osteochondrosarcoma, and in experiment 12, from a compact endothelial growth with no evidence of bone or cartilage formation.

After the first passages, leukocytes disappeared from the cultures, which were composed of polygonal stout fibroblast-like cells, with no evidence of cartilage or bone formation. The appearance of these cells in the fourth and ninth generation of experiment 12, in which the inoculations were most successful, is illustrated in figure 6 *B, C* and *D*. They did not possess the pale nuclei with large nucleoli

TABLE 10.—*Inoculations with Cultures of Tumors Produced by Virus 12*

Experiment	Age of Culture, Days	Passage	Route of Injection*	Chickens Given Injections	Successful Injections	Type of Disease Produced†
10	Immediate	Original.....	I.m.	5	3	2 S, 1 SL
	23	1st and 2d.....	I.m.	3	0	.....
	25	2d and 3d.....	I.v. and I.m.	3	0	.....
11	5	Original.....	I.v. and I.m.	5	3	1 SL, 2 L
	18	Original and 1st....	I.m.	4	0	.....
		1st.....	I.m.	1	1	1 L‡
	38	Original, 1st, 2d, 3d	I.m.	2	0	.....
		4th.....	I.m.	3	1	1 L‡
12	Immediate	Original.....	I.m.	2	2	2 SL
	38	1st to 5th.....	I.v. and I.m.	3	2	2 S
	71	2d to 7th.....	I.v. and I.m.	4	0	.....
	91	7th to 9th.....	I.v. and I.m.	3	1	1 SL
13	Immediate	Original.....	I.m.	5	3	3 S
	25	1st to 3d.....	I.v. and I.m.	4	1	1 L
	58	3d to 4th.....	I.v. and I.m.	4	2	1 L, 1 L‡

* I.m. signifies intramuscular; I.v., intravenous.
† S signifies osteosarcoma; L, leukosis.
‡ In these cases of lymphomatosis the predominant type of cell was the small lymphocyte, and it is uncertain whether the disease was produced by the virus of strain 12.[16]

characteristic of the cells of cultures of virus 2. The cells grown in experiment 11 (fig. 6 *A*) showed, however, some similarity to these cells. The morphologic differentiation of these cells from normal fibroblastic cells requires further study. Silberberg,[15] reviewing recently tissue culture studies of tumors, has come to the conclusion that there are only quantitative differences between tumor and normal cells.

The results of inoculations indicate that these cells possess the ability to produce both leukosis and bone tumors (table 10).

Previous experiments have shown that the results of inoculations with virus 12 are variable. Since the incidence of spontaneous osteochondrosarcoma and leukosis produced by virus 2 is negligible, the successful inoculations are of greater significance than the unsuccessful ones. The table shows that virus 12 was present in the

15. Silberberg, M.: Arch. Path. **21**:663, 1936.

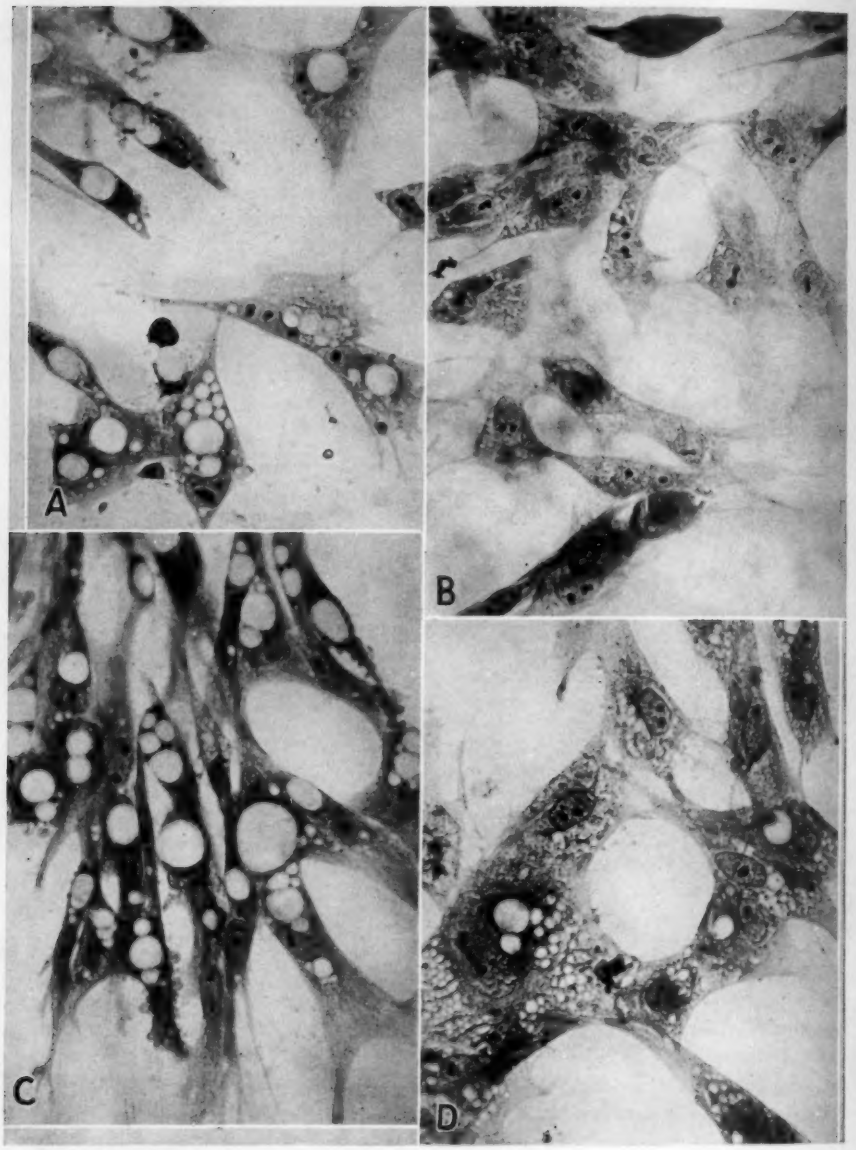

Fig. 6.—*A* represents an eight day growth of the fifth passage of sarcoma produced by virus 12 (experiment 11); *B*, a three day growth of the fourth passage (experiment 12); *C*, a four day growth of the same passage as *B*, and *D* a three day growth of the ninth passage of the same culture—all × 500.

cultures in experiment 12 after ninety-one days of incubation and in experiment thirteen after fifty-eight days of incubation. In experiment 10 the chickens that received injections of twenty-three and twenty-five day cultures remained healthy. Experiment 11 is difficult to interpret because the two cases of lymphomatosis which occurred among the chickens given injections of the tissue cultures resembled the common variety of lymphomatosis, which is not produced by virus 12.[16]

It is noteworthy that the ninety-one day culture in experiment 12 produced both sarcoma and leukosis, whereas the seventy-one day culture was unsuccessful.

Summary of Results with Virus 12.—Virus 12 was maintained in vitro for ninety-one days by cultivating the growth produced by this virus. The cells grown in vitro resembled fibroblasts, osteogenesis was not observed, and blood cells were not found in the cultures. When the cultures were inoculated into animals, they produced both osteochondrosarcoma and leukosis.

TABLE 11.—*Inoculations with Cultures of Spleen Particles from a Normal Chicken, Grown in the Presence of Virus 13*

Age of Culture, Days	Passage	Chickens Given Injections	Successful Injections*	Length of Life After Injection, Days	
				Successful Injections†	Unsuccessful Injections†
Immediate	Original	3	2 SE 1 S	K, 42, 52 D, 41	.....
9	Original and 1st	4	0	.....	K, 68, 68, 68, 68
41	1st, 2d, 3d, 5th	4	0	.....	K, 67, 67, 67; K, 68‡

* S signifies sarcoma; E, erythroleukosis.
† K indicates that the chickens were killed; D, that they died.
‡ This chicken had aleukemic lymphomatosis.

CONTROL OBSERVATIONS

The experiments with viruses 1 and 2 described in the preceding sections showed that it is possible to cultivate viruses producing leukosis or sarcoma by growing the neoplastic cells produced by them. The cells grown from tumors produced by viruses 13 and 12 were probably also neoplastic, but we were not able to distinguish them from normal cells. The following experiments were undertaken to determine whether these viruses multiply in the presence of cells grown from normal tissues.

Survival of Virus 13 in the Presence of Normal Cells.—In the experiment shown in table 11 fragments of the spleen of a healthy 4 week old chicken were cultured with virus 13. The virus was obtained by freezing rapidly to —31 C. tumor tissue produced by this virus. It is not certain that all neoplastic cells had been destroyed by freezing, but thus far we have never been able to demonstrate by means of tissue culture the presence of living cells in frozen material. Moreover, after freezing, the material was centrifugated at 2,000 revolutions per minute

16. Furth, J., and Breedis, C.: Arch. Path. **20**:379, 1935.

during ten minutes, and the bottom layer was discarded. The supernatant fluid was used in the preparation of the tissue cultures, in place of normal Tyrode solution.

The cultures were composed of fibroblastic cells (fig. 3 *G*) which we could not distinguish from either the sarcoma cells of virus 13 or normal fibroblasts. The cultures that were injected into chickens immediately after they were prepared produced sarcoma in all three inoculated birds, in two of which leukosis also developed. All injections were intramuscular. The chickens given injections of the nine and forty-one day cultures remained healthy (table 11).

Similar results were obtained in the experiments recorded in table 12. In this experiment the leg of a 7 day old chick embryo was cut up in the presence of virus 13, which was obtained in the manner described in the preceding experiment. Sarcoma and leukosis were produced by the six and eleven day cultures, but inoculations with older cultures were unsuccessful.

TABLE 12.—*Inoculations with Cultures of Legs of a 7 Day Old Chicken Embryo, Grown in the Presence of Virus 13*

Age of Culture, Days	Passage	Material Injected	Route of Injection*	Chickens Given Injections	Successful Injections†	Length of Life After Injection, Days: Successful Injections‡	Length of Life After Injection, Days: Unsuccessful Injections‡
6	Original	Supernatant fluid Culture particles	I.v. I.m.	2	1 S	D, 52	K, 78
11	1st	Culture particles	I.m.	2	1 SE	D, 40	K, 81
33	5th, 6th	Supernatant fluid Culture particles	I.v. I.m.	3	0	.....	K, 95, 95, 95
51	6th, 7th, 8th	Supernatant fluid Culture particles	I.v. I.m.	3	0	.....	K, 86, 100, 100
74	9th, 10th	Supernatant fluid Culture particles	I.v. I.m.	6	0	.....	K, 69, 71, 99, 99, 99, 99

* I.v. indicates intravenous; I.m., intramuscular.
† S signifies sarcoma; E, erythroleukosis.
‡ K indicates that the chickens were killed; D, that they died.

Summary of Results with Normal Cells.—These experiments indicate that virus 13 dies in the presence of normal fibroblastic cells of embryonal leg and adult spleen.

Survival of Virus 1 in the Presence of Normal Cells.—A study of the survival of virus 1 in the presence of normal cells was made in one experiment. Since the inoculations made with the cultures immediately after their preparation were unsuccessful, these experiments will not be described in detail; although only two of the chickens inoculated with the immediate cultures lived longer than one month, ten of those inoculated with from five to twenty-one day cultures survived this period without showing any evidence of leukosis.

Survival of Virus 1 in the Presence of Sarcoma Cells.—Since pure cultures of sarcoma cells of virus 13 produced leukosis, experiments were undertaken to determine whether a pure virus of leukosis would survive in the presence of common sarcoma cells.

In these studies virus 1, which does not produce sarcoma, was used. The sarcoma cells were those of virus 11, which does not produce leukosis.

In one experiment the sarcoma cells produced by virus 11 were grown in the plasma of a leukotic chicken. Of sixteen chickens inoculated at various intervals during the forty-three days of cultivation of the sarcoma cells, nine presented sarcoma and none leukosis. The presence of the virus of leukosis in this plasma was not determined, but nine of ten chickens inoculated with 0.02 cc. of whole blood of the leukotic chicken died of leukosis.

A second experiment has already been described in a previous communication.[7d] Sarcoma of a chicken that also had leukosis was grown in homologous plasma. Subpassages were made in normal plasma. The cultures consisted of spindle-shaped and fibroblast-like cells. Subcultures were made at from four to six day intervals in plasma and serum of normal chickens. The results of inoculations with these tissue cultures were as follows: Of twenty chickens given injections

TABLE 13.—*Inoculations with a Mixed Culture of Sarcoma Produced by Virus 11 and Leukotic Spleen Produced by Virus 1*

Age of Culture, Days	Passage	Material Injected	Route of Injection*	Chickens Given Injections	Successful Injections†	Length of Life After Injection, Days: Successful Injections‡	Length of Life After Injection, Days: Unsuccessful Injections‡
Immediate	Original	Culture particles	I.m. and I.p.	3	0	.....	D, 73; K, 91, 91
		Supernatant fluid	I.v.	3	2 E	D, 34; K, 111	K, 91
30	1st, 2d, 3d	Culture particles	I.m. and I.p.	3	2 S 1 SM	D, 50, 51 K, 32	....
		Supernatant fluid Culture particles	I.v. I.m.	3	2 S 1 S	D, 52; K, 55 K, 53	....
50	3d	Supernatant fluid Culture particles	I.v. I.m.	4	4 S	D, 47, 59, 59, 82	.....
	4th, 5th	Supernatant fluid Culture particles	I.v. I.m.	4	4 S	D, 46, 50 K, 18, 66	....
61	6th, 7th	Supernatant fluid Culture particles	I.v. I.m.	4	4 S	D, 30, 32 K, 44, 44	....

* I.v. indicates intravenous; I.m., intramuscular; I.p., intraperitoneal.
† E signifies erythroleukosis; S, sarcoma; M, myeloid leukemia.
‡ K indicates that the chickens were killed; D, that they died.

at various intervals during the thirty-three days of cultivation in vitro, all but one presented sarcoma and none leukosis.

In a third experiment a mixed culture was set up with sarcoma produced by virus 11 and spleen of a chicken having myeloid leukosis of virus 1. When this experiment was planned, it was not known that a few leukotic cells may multiply in the culture of a leukotic spleen during several weeks and that such cultures produce leukosis. No attempts were made to ascertain the presence of myeloblasts in these cultures.

Table 13 shows that the inoculations with this culture produced leukosis and sarcoma after thirty days of cultivation. The fifty and sixty-one day cultures produced sarcoma in all of the twelve inoculated chickens, none of which had leukosis. The cells of these cultures are illustrated in figure 3 *H*.

Summary of Results with Sarcoma Cells.—These experiments indicate that leukotic virus 1 does not survive in the presence of common sarcoma cells.

SUMMARY AND CONCLUSIONS

Leukemic myeloblasts of chickens (virus 1) remain viable in vitro in liquid cultures for at least thirty days and produce leukosis when inoculated into chickens.

In solid cultures of spleen and bone marrow from chickens with myeloid leukemia a symbiotic growth of myeloblasts and fibroblast-like cells occurs. These cultures likewise have the ability to produce leukosis.

Fibroblast-like cultures of bone marrow of a chicken with erythroleukosis, not containing leukotic blood cells, failed to produce leukosis.

The leukotic virus 2 survives in vitro for at least thirty-six days in the presence of cells resembling neoplastic endothelium produced in vivo by this virus.

Osteochondrosarcoma produced by virus 12 when grown in vitro yields fibroblast-like cells. Inoculation of these cells into chickens after ninety-one days of cultivation produced both osteochondrosarcoma and leukosis. Virus 12 produces both osteochondrosarcoma and leukosis.

Cultures of sarcoma produced by virus 13, grown in vitro for one hundred and fifty-eight days and free from blood cells, produced both sarcoma and leukosis. This observation supports the view that virus 13 stimulates both primitive blood cells and mesenchymal cells other than blood cells.

Virus 13 perishes in vitro within approximately two weeks in the presence of normal fibroblastic cells of spleen and of embryonal leg.

Virus 1, which produces leukosis, perishes in the presence of sarcoma cells produced by a virus that does not stimulate primitive blood cells.

These experiments support the opinion that oncogenic viruses multiply in vitro only in the presence of cells on which they confer neoplastic properties. They indicate that a single virus may stimulate both primitive blood cells and fibroblast-like cells.

The different viruses studied retained their characteristics during a period of observation of from three to five years.

CHYLOUS EFFUSIONS PRODUCED BY EXPERIMENTAL LIGATION OF THE SUPERIOR VENA CAVA

CHEMICAL AND CYTOLOGIC STUDIES

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Chylothorax, while not a common condition in man, occurs with sufficient frequency to make a study of it both interesting and important. In the literature we have found references to one hundred cases in which the diagnosis seems to have been correct. In the reports dealing with eighty-three of these cases, which we have carefully examined, the authors have given very meager data on the cellular content of the fluid and on the changes in the cell picture in the blood stream. Although somewhat more complete, the chemical studies on these fluids have also been inadequate. Therefore, it has seemed wise to present a brief report of the findings in these cases, with the hope that others, which may from time to time be discovered, will be studied more completely.

Thirteen differential blood counts were made in ten of the reported cases of chylothorax (table 1). The total white blood cell counts varied from 4,000 to 25,000. In six instances there was a high proportion of lymphocytes, while in seven the lymphocytes varied from 6 to 16 per cent.

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In a preliminary paper [1] we have reported what we believed to be the first experimental production of typical chylothorax. This condition was produced in twenty-one of thirty-six animals (dogs and cats) in each of which the superior vena cava was ligated. The present report is a continuation of that study, with particular emphasis on the chemical and cellular composition of the fluids. The cellular changes occurring in the blood stream as a result of the ligation of the superior vena cava

TABLE 1.—*Differential Counts on Human Chylous Effusions*

Author	Red Blood Cells	White Blood Cells	Lympho-cytes	Neutro-phils	Eosino-phils	Epi-thelial	Endo-thelial	Phago-cytes	Chylo-microns
Sale, L.: Interstate M. J. **19**: 50, 1912	.....	Few	100%	.....	.....	.....	.....	.....	.....
Malone, A. E., and Waldrop, J. R.: Lancet **1**: 1116, 1919	.....	.....	Practically 100%	.....	.....	.....	.....	.....	.....
Fürth, E.: Wien. klin. Wchnschr. **40**: 350, 1927	Few	.....	Many	.....	.....	.....	.....	Many	Present
Sherman [6]	Occasional	.....	Many	Occasional	.....	Occasional	.....	.....	Present
Pisek, G. R.: Arch. Pediat. **34**: 929, 1917	.....	12,160	95%	.....	Few	.....	.....	.....	Present
Watts, S. H.: Ann. Surg. **74**: 691, 1921	.....	.....	Many	Predominant	.....	.....	.....	.....	.....
Paltre, M.: Bull. et mém. Soc. nat. de chir. **55**: 813, 1929	.....	.....	28% (mononuclears)	72%	.....	.....	Abundant	.....	.....
Outland, J. H., and Clendenning, L.: J. A. M. A. **66**: 1833, 1916	Many	Many	.....	.....	.....	.....	.....	.....	Present
Andrews, C. F.: Nebraska M. J. **14**: 26, 1929	Few	.....	Few	.....	.....	.....	.....	.....	Present
MacNab, D. S., and Scarlett, E. P.: Canad. M. A. J. **27**: 29, 1932	.....	.....	Few	Few	.....	.....	.....	.....	Present
Dietze, A.: Deutsche Ztschr. f. Chir. **73**: 450, 1904	Few	Few	.....	.....	.....	.....	.....	.....	.....
Williamson, C. S.: Internat. Clin. **4**: 7, 1920	Occasional	None	.....	.....	.....	.....	.....	.....	.....
Remé, G.: Monatschr. f. Kinderh. **34**: 135, 1926	.....	.....	.....	.....	.....	.....	.....	.....	Present
Jennings, C. G., and Rich, H. M.: Arch. Pediat. **25**: 195, 1908	.....	.....	.....	.....	.....	.....	.....	.....	Present

are compared with the cellular picture of the chylous fluids. We have to date ligated the superior vena cava in forty-seven animals (thirty-six of these were reported in the paper referred to [1]; eleven additional animals have been studied since that time). In twenty-eight animals of this group typical chylous effusions developed; in eleven fluid was obtained from the pleural cavities which was, however, not chylous, and in eight animals no fluid developed. In all of these experiments in which the superior vena cava was ligated, it is assumed that the develop-

1. Blalock, Alfred; Cunningham, R. S., and Robinson, C. S.: Ann. Surg. **104**:359, 1936.

ment of chylous effusions was dependent on the obstruction thus produced to the delivery of lymph from the abdominal viscera through the thoracic duct and its collateral and anastomotic channels.

CYTOLOGIC STUDIES

Since we have no experimental results with which to compare our findings, it becomes necessary to turn to the cases in man. In only twelve instances has there been an attempt to analyze the cellular characteristics of chylous fluids (table 2). The reports are disappointing

TABLE 2.—*Differential Counts on Blood in Cases of Chylous Effusion*

Author	Red Blood Cells	White Blood Cells	Neutrophils	Eosinophils	Basophils	Lymphocytes	Transitionals and Mononuclears	Unclassified Cells
Normal human blood.....	5,000,000	7,000	5,657	363	90	1,629	471 (monocytes)	..
Sherman*	4,870,000	9,000	5,670	45	0	3,285	0	..
	5,160,000	10,850	5,750	325	0	4,774	0	..
Sale, L.: Interstate M. J. **19**: 50, 1912	4,900,000	4,800	2,976	48	48	1,536	192	..
Lewin, P.: Am. J. M. Sc. **152**: 71, 1916	4,600,000	18,760	6,378*	94	187	11,625	18†	187
Tuley, H. E., and Graves, S.: J. A. M. A. **66**: 1844, 1916..	4,900,000	10,000	8,200	70	0	950	150§	..
	4,890,000	9,520	8,378	19	0	1,094	38§	..
	4,800,000	25,200	23,998	0	0	2,419	0	..
Malone, A. E., and Waldrop, J. R.: Lancet **1**: 1116, 1919..	3,420,000	13,800	9,660	0	0	2,898	1,182	..
Williamson, C. S.: Internat. Clin. **4**: 7, 1920.....	.....	9,100	6,461	0	91	2,366	182	..
Lyter, J.: M. Clin. North America **11**: 479, 1927.....	4,480,000	4,600	3,867	0	0	736	0	..
Fürth, E.: Wien. klin. Wchnschr. **40**: 350, 1927.....	4,320,000	8,500	6,205	85	0	1,870	170§	..
Van Nuys, R. G.: California & West. Med. **34**: 269, 1931....	.....	7,300	4,453	0	0	2,701	146§	..
Yater, W. M.: Ann. Int. Med. **9**: 600, 1935	4,870,000	8,600	6,536‡	86	0	1,720	516	..

* This count represents polymorphonuclears plus neutrophilic myelocytes.
† Indentate nuclei were shown.
§ Transitional forms alone were present.
‡ Polymorphonuclears include 24 per cent "band cells"; 3 per cent young cells are not tabulated.

in that no complete counts (total and differential) were made. The rough estimations made in certain cases have agreed in that the majority of the investigators noted a predominance of lymphocytes.

In table 3 we present the total differential counts on the fluids obtained from the cats and dogs in which we produced true chylothorax. These counts are averages. The number of counts from which each average was obtained is shown in parenthesis following the total count. The variation in the total number of cells present and in the proportions of the various elements has been very wide. The general average of all animals showed a total count of 20,000 cells, 2,000 neutrophils and 17,500 lymphocytes. The cellular composition of each individual fluid used for chemical studies is given in table 4.

TABLE 3.—*Averages of Cell Counts on Fluids from Chests of Animals in Which True Chylothorax Had Been Produced*

Animal	White Blood Cells*	Neutrophils	Eosinophils	Lymphocytes	Monocytes	Clasmatocytes	Serosal Cells	Chylomicrons
Cat 10	40,880 (2)	672	0	36,335	0	0	0	+
Cat 11	15,900 (4)	624	0	14,928	0	0	0	++
Cat 12	22,145 (4)	836	839	20,020	449	0	0	0
Cat 13	9,349 (7)	2,595	344	5,554	547	85	73	++
Cat 19	13,650 (4)	3,061	240	9,861	429	60	0	+++
Cat 20	10,554 (13)	4,447	223	5,387	327	139	31	++
Cat 44	26,000 (2)	588	89	25,029	171	122	0	0
Cat 46	48,400 (1)	2,420	726	45,254	0	0	0	+++
Cat 47	30,000 (2)	2,420	1,128	26,214	238	0	0	0
Cat 48	23,400 (3)	1,058	210	22,342	122	0	0	++++
Cat 49	21,571 (11)	1,042	161	20,153	1,851	40	0	+
Cat 50	28,411 (7)	1,128	198	26,663	139	211	0	0
Cat 51	12,800 (2)	1,621	265	10,464	362	0	22	+
Cat 52	23,000 (10)	3,336	621	18,546	341	85	70	++++
Cat 53	11,024 (11)	1,575	611	8,531	269	6	2	+
Cat 54B	12,000 (2)	3,600	150	7,770	390	0	90	++
Cat 54A	20,855 (16)	2,973	0	16,488	0	0	0	+
Cat 56	15,144 (11)	5,914	246	8,233	496	206	39	+++
Cat 79	17,213 (3)	1,117	227	15,244	623	16	0	++
Cat 87	11,057 (10)	1,766	180	7,357	342	55	0	+++
Average	20,668	2,140	323	17,519	354	51	16	
Dog 26	19,500 (4)	14,297	35	3,870	1,108	0	187	++
Dog 31	11,109 (16)	3,834	136	5,246	1,431	191	26	++
Dog 83	11,830 (9)	3,527	7	7,143	590	87	318	+
Dog 128	13,000 (2)	9,815	0	1,950	1,105	65	65	0
Average	13,859	7,868	45	4,552	1,058	86	149	

* The number of counts is given in parentheses.

TABLE 4.—*Cell Composition of Samples Used for Chemical Studies*

Animal	Date	White Blood Cells	Neutrophils	Eosinophils	Lymphocytes	Monocytes	Clasmatocytes	Serosal Cells	Chylomicrons
Cat 13	7/29/35	10,400	1,768	416	7,280	208	520	208	10
	8/ 3/35	10,400	1,248	312	8,216	624	0	0	10
Dog 26	11/22/35	7,600	2,166	0	3,230	1,558	0	646	0
	12/ 5/35	27,600	16,560	138	10,212	690	0	0	Few
	12/20/35	37,600	37,224	0	374	0	0	0	15
Cat 20	8/ 3/35	6,800	1,632	0	4,420	544	204	0	15
	8/13/35	16,400	12,300	328	2,952	328	164	328	15
	8/16/35	7,200	2,916	72	3,456	648	36	72	15-20
	8/26/35	9,920	4,364	99	4,464	594	396	0	15
	9/ 2/35	9,840	7,970	0	1,870	0	0	0	0
Cat 44	11/29/35	16,400	820	0	15,170	164	246	0	0
Cat 48	12/16/35	24,400	1,952	122	21,960	366	0	0	20
Cat 49	12/ 9/35	18,800	1,692	94	16,638	376	0	0	Moderate number
	12/13/35	22,800	912	0	21,774	114	0	0	5
	12/23/35	17,600	1,408	0	16,192	0	0	0	0
	1/10/36	12,400	992	124	10,768	248	248	0	Occasional
	1/13/36	11,200	1,232	224	9,632	112	0	0	0
Cat 50	12/ 9/35	27,200	1,632	272	24,752	544	0	0	0
	1/10/36	22,400	560	224	21,280	336	0	0	0
Cat 52	12/13/35	27,600	4,278	138	23,184	0	0	0	20
	1/10/36	15,200	1,900	228	12,236	836	0	0	Immense number
	1/24/36	16,400	4,920	574	10,906	0	0	0	20
	2/ 7/36	12,400	3,100	1,612	6,448	372	620	248	15
Cat 53	12/ 2/35	3,200	768	0	2,384	16	16	16	10
	12/ 6/35	11,200	1,232	224	9,632	112	0	0	0
	12/23/35	9,200	2,464	644	5,754	274	0	0	0
	1/10/36	10,800	2,430	2,052	5,886	216	0	0	0
Cat 54	11/23/35	6,000	2,520	120	3,120	60	0	180	0
	11/25/35	18,000	4,680	180	12,420	720	0	0	Many
Cat 56	12/13/35	20,800	10,400	0	10,400	0	0	0	0
	12/16/35	28,400	2,272	284	23,714	0	2,130	0	15
	12/18/35	20,800	7,384	936	12,168	312	0	0	15
	12/20/35	15,600	10,452	468	4,680	0	0	0	10

It will be noticed that in the majority of the experiments there was a great preponderance of lymphocytes, although there were usually considerable numbers of neutrophils. It is obvious that as the total number of cells increases there is a larger proportionate number of lymphocytes. The general predominance of lymphocytes and particularly the predominance of fluids with the higher counts indicate, we believe, the

TABLE 5.—*Blood Cell Changes in Animals Selected as Typical of Each Group*

Group*	Lympho-cytes	Eosino-phils	Mono-cytes
Group 1 (7 animals)			
Cat 44-A			
Preliminary (5 counts)	3,073	1,880	348
After ligation of superior vena cava (13 counts)	1,555	913	727
Cat 54-B			
Preliminary (5 counts)	3,066	3,326	759
After ligation of superior vena cava (7 counts)	839	260	1,407
Group 2 (1 animal)			
Cat 45-A			
Preliminary (3 counts)	5,012	1,129	747
After ligation of superior vena cava (7 counts)	2,016	375	629
Group 3 (6 animals)			
Cat 87-B			
Preliminary (1 count)	3,010	1,032	516
After ligation of superior vena cava (23 counts)	1,362	1,710	631
Group 4 (no animals)			
Group 5 (4 animals)			
Cat 56-A			
Preliminary (5 counts)	1,939	303	404
After ligation of superior vena cava (7 counts)	1,490	247	924
Group 6 (5 animals)			
Cat 1			
Preliminary (4 counts)	1,547	992	446
After ligation of superior vena cava (10 counts)	2,037	1,048	525
Group 7 (1 animal)			
Cat 54-A			
No preliminary counts			
No counts until 3 months			
After ligation of superior vena cava (7 counts)	2,908	266	798

* The groups were as follows:
Group 1. Cats in which there was a consistent drop in eosinophils and lymphocytes with an accompanying rise in monocytes.
Group 2. Cats in which there was a constant drop in lymphocytes and eosinophils but no accompanying change in monocytes.
Group 3. Cats in which there was a drop in lymphocytes with no consistent change in other cells.
Group 4. Cats in which there was a drop in eosinophils with no consistant change in other cells. This group is included in order that this table may be compared with table 5 in the paper by Blalock, Robinson, Cunningham and Gray.[2]
Group 5. Cats in which there was a rise in monocytes with no consistent change in other cells.
Group. 6. Cats in which there were no marked changes in the leukocytes.
Group 7. Miscellaneous.

lymphatic origin of these fluids. We have found further that following ligation of the superior vena cava there is, in a large proportion of the animals, a drop in the number of circulating eosinophils. This drop in eosinophils is quite comparable to the similar observation in the animal in which the thoracic duct has been obstructed.[2] The importance of this finding has been discussed in the paper referred to [1] and need not

2. Blalock, Alfred; Robinson, C. S.; Cunningham, R. S., and Gray, Mary E.: Arch. Surg. **34**:1049, 1937.

be dealt with here. In our experiments the results of ligation of the superior vena cava have been quite uniform. We have divided these experiments into seven groups, and these are shown in table 5. In each group the averages for a typical animal are given, showing the characteristic fall in lymphocytes and eosinophils which followed the operative procedure. There was a marked rise in neutrophils in the majority of instances.

CHEMICAL STUDIES

Although considerable excellent work has been done on tissue fluids with respect to their electrolyte and protein contents, their lipoidal constituents have been studied as casually as have the cell pictures. There are many isolated analyses of fluids for cholesterol, fat, ether-soluble constituents, and other constituents, but even these were, in many cases, done under circumstances that make their accuracy doubtful. Table 6 gives the figures for the total lipid distributions, determined by the methods described by Kirk, Page and Van Slyke,[3] for thirty-three of the fluids produced by ligation of the superior vena cava in cats and dogs. Some of these fluids were clear, but most of them were milky.

Several classifications have been proposed for fluids such as we have produced. These include the use of such terms as "nonchylous" for clear, serous fluids; "chylous" for fluids which are milky because of the presence of fat from the chyle which has contributed to their formation; "pseudochylous" for fluids which are milky because of the presence of substances other than fat, and "chyliform" for fluids which are milky from the presence of fat produced by the destruction of cellular elements in the fluid.

Although the assumption of the existence of the last two is entirely plausible, the evidence that any of the examples described in the literature has been accurately classified is rather dubious. For instance, Wallis and Schölberg [4] reported a thorough study of four fluids which they classified as pseudochylous, the milkiness of which they ascribed to a lecithin-globulin complex. Nevertheless, some fat was known to have been present, and subsequently doubt was cast on their method for the quantitative measurement of the fat, so that it may have been adequate in amount to account for the production of opacity. Blankenhorn [5] claimed that all of the phenomena ascribed to the agency of other substances can be explained by the behavior of colloidal fat and that the term "pseudochylous" is unnecessary, but he gives no experi-

3. Kirk, E.; Page, I. H., and Van Slyke, D. D.: J. Biol. Chem. **106**:203, 1934.

4. Wallis, R. L. M., and Schölberg, H. A.: Quart. J. Med. **3**:301, 1909-1910; **4**:153, 1910-1911.

5. Blankenhorn, M. A.: Arch. Int. Med. **32**:140, 1923.

mental results. Since fat-free biologic fluids are known the milky appearance of which is due to substances other than fat, e. g., fat-free milk, the possible existence of transudates of this character must be admitted.

TABLE 6.—*Chylous Fluids*

		Determined						Calculated					
				Nitrogen		Cholesterol							
Animal	Date	Total Carbon	Total Phosphorus	Total	Amino	Total	Free	Esterified	Esters*	Phosphatides†	Neutral Fats‡	Total Lipids§	
												I	II
Cat 13	7/29	921	0.0	11.3	2.1	81.3	26.6	54.7	92.4	0.0	1,085	1,204	1,197
	8/ 3	341	0.0	8.9	1.4	75.7	27.8	47.9	81.0	0.0	331	440	443
Dog 26	11/22	213	1.2	8.3	1.0	66.5	30.4	36.1	61.0	28.2	156	276	277
	12/ 5	341	0.9	9.6	0.8	30.6	28.0	2.6	4.4	21.1	396	450	443
	12/20	2,046	6.8	16.6	4.9	81.1	79.9	1.2	2.0	159.8	2,471	2,713	2,660
Cat 20	8/ 3	1,838	2.7	16.1	6.7	128.6	54.1	74.5	125.9	63.4	2,173	2,416	2,389
	8/13	3,744	19.5	18.6	13.1	165.0	51.7	113.3	191.5	458.3	4,276	4,978	4,867
	8/16	2,976	14.5	24.7	8.3	155.8	50.3	105.5	178.3	340.8	3,381	3,950	3,869
	8/26	1,840	10.9	12.4	6.5	152.4	31.2	121.2	204.9	256.2	1,947	2,439	2,392
	9/ 2	3,223	5.2	24.4	9.7	80.0	51.5	28.5	48.2	122.2	4,038	4,200	4,190
Cat 44	11/29	201	1.9	17.3	1.1	68.5	51.4	17.1	28.9	44.7	138	263	261
Cat 48	12/16	2,295	10.7	23.7	8.2	121.5	84.6	36.9	62.4	251.5	2,648	3,047	2,984
Cat 49	12/ 9	772	1.9	21.2	5.4	79.7	45.6	34.1	57.6	44.7	867	1,015	1,004
	12/13	653	2.1	18.4	2.5	100.9	34.9	66.0	111.5	49.4	658	854	849
	12/23	120	0.5	12.9	1.1	48.2	29.7	18.5	31.3	11.8	81	154	156
	1/10	140	2.0	15.6	1.6	25.0	22.1	2.9	4.9	47.0	114	188	182
	1/13	155	2.2	13.6	0.7	43.8	17.9	25.9	43.8	51.7	92	205	202
Cat 50	12/ 9	169	3.4	20.5	0.7	52.4	20.7	31.7	53.6	79.9	72	226	220
	1/10	134	1.2	20.5	0.5	42.0	12.8	29.2	49.3	28.2	84	174	174
Cat 52	12/13	2,614	18.5	16.1	8.8	143.7	77.2	66.5	112.4	434.8	2,864	3,488	3,398
	1/10	4,125	7.0	21.3	11.7	154.5	82.4	72.1	121.8	164.5	5,077	5,446	5,363
	1/24	1,900	8.3	9.5	5.9	118.4	58.9	59.5	100.6	195.1	2,163	2,518	2,470
	2/ 7	1,949	19.7	18.9	12.4	196.7	76.9	119.8	202.5	463.0	1,863	2,605	2,534
Cat 53	12/ 2	1,234	5.0	10.3	6.4	120.2	55.1	65.1	110.0	117.5	1,345	1,628	1,604
	12/ 6	419	1.0	7.9	4.2	75.5	45.8	29.7	50.2	23.5	427	547	545
	12/23	...	13.9	5.9	2.5	63.8	20.4	43.4	73.3	326.7	.....	.....	.....
	1/10	900	1.0	6.2	0.9	46.6	18.0	28.6	48.3	23.5	1,095	1,185	1,170
Cat 54	11/23	930	3.8	21.2	8.2	132.1	57.1	75.0	126.8	89.3	948	1,221	1,209
	11/25	1,533	6.5	19.2	8.3	158.4	57.1	101.3	171.2	152.8	1,640	2,021	1,993
Cat 56	12/13	1,281	7.2	20.1	8.6	169.3	50.5	118.8	200.8	169.2	1,208	1,689	1,665
	12/16	2,160	19.5	19.0	12.1	174.3	82.1	92.2	155.8	458.3	2,191	2,887	2,808
	12/18	1,778	7.5	8.8	4.4	119.3	55.5	63.8	107.8	176.3	2,014	2,354	2,311
	12/20	1,092	4.0	12.4	6.4	133.5	67.3	66.2	111.9	94.0	1,163	1,436	1,420

* Esterified cholesterol × 1.69.
† Lipid phosphorus × 23.5.
‡ 1.32 total calcium — [(15.5 × phosphorus) + 0.839 (free cholesterol) + 1.4 (esterified cholesterol)].
§ I = phosphatides + free cholesterol + cholesterol esters + neutral fats; II = 1.3 × total calcium.

All of our fluids contained fat, though in several the quantity was small. These, however, were the clear, nonchylous fluids. All of those which were milky contained considerable fat. Unfortunately their opacity was not accurately measured, but their appearance described merely as "slightly cloudy," "milky" or "very milky." Dividing these into three groups, clear, milky and very milky, one finds a close rela-

tionship between the degree of opacity and the fat content. This makes possible the definite classification of our milky fluids as chylous fluids.

A survey of the analytic results reported in the literature indicates a wide range in the lipid composition of chylous fluids, as might be anticipated from a consideration of the methods of their formation. Fluids of nontraumatic origin contain only such lipid material as may pass through the walls of the blood vessels, lymphatics or other membranes. Those of frank traumatic origin, owing to actual openings in the wall of the thoracic duct or of the ruptured lymphatics, may, unless diluted with other fluid, contain as much lipid as the fluids in these vessels, which, of course, may be great. This type of fluid may also be produced by rupture of smaller lymph vessels due to increased intralymphatic pressure in a fashion not easily recognized. Hence, results in the literature refer to fluids varying from almost pure postprandial chyle to slightly cloudy transudates.

Wallis and Schölberg [4] observed an increase in the fat content of fluids with each succeeding withdrawal. Sherman,[6] on the other hand, found a decrease in fat content. Our fluids exhibited no consistent behavior, the fat contents fluctuating irregularly with successive tappings.[1] We explain these fluctuations in the composition of our fluids by the assumption, indicated previously, that our fluids arose from the prevention of the delivery of lymph into the venous circulation. The resulting dilatation of the lymph vessels produced variable increases in permeability and perhaps intermittently ruptures, which at times discharged pure chyle into the accumulating fluid, thus increasing its lipid content.

The analytic data for our cases are shown in table 6. In this table total lipids have been calculated by both of the methods discussed by Kirk, Page and Van Slyke.[3] Although the agreement is not quite so good as Page and his co-workers [7] found for blood plasma, it is still so close to the limits of accuracy of the methods of analysis as to make either one satisfactory for fluids as well as for blood.

COMPOSITION OF FLUID LIPIDS

The ranges in the composition of our fluids, divided into three groups according to opacity, are shown by the figures in table 7. These are the means and standard deviations of the means. For comparison, the corresponding values for normal human plasma as found by Page and his associates [7] and some other figures are also given. The greatest percentage deviations are in the neutral fats and total lipids of the

6. Sherman, DeWitt H.: Arch. Pediat. **24**:646, 1907.

7. Page, I. H.; Kirk, E.; Lewis, W. H.; Thompson, W. R., and Van Slyke, D. D.: J. Biol. Chem. **111**:635, 1935.

clear fluid group, but this is due to one fluid (C53, 1/10), which, because of the small number of samples, produced a noticeable effect. This, of course, could not be discarded. It was described as a clear fluid independently by two observers and shows that in borderline cases some clear fluids may have more fat than others which are milky. In other words, as has been pointed out by others, not only the total fat content but its physical condition is a factor in the production of lactescence. The increase in all lipid constituents with increase in

TABLE 7.—*Average Composition of Fluids*

Fluid	Sample	Total Lipid, Mg. per 100 Cc.	Neutral Fat, Mg. per 100 Cc.	Cholesterol, Mg. per 100 Cc.		Phosphatide, Mg. per 100 Cc.	Total Nitrogen, Mg. per 100 Cc.	Amino-nitrogen, Mg. per 100 Cc.
				Total	Free			
Clear fluids.....	8	342 ± 373*	239 ± 378†	49 ± 13	24 ± 12	77 ± 103‡	14.1 ± 5.7	2.2 ± 2.4
		100	70	14	7	22		
Chylous A#.....	15	1,363 ± 801	1,111 ± 669	110 ± 41	48 ± 15	114 ± 117	14.2 ± 5.2	5.2 ± 3.3
		100	80	8	4	8		
Chylous B#.....	10	3,544 ± 1,077	3,073 ± 1,087	137 ± 36	65 ± 18	285 ± 131	18.6 ± 5.7	9.0 ± 7.9
		100	87	4	2	8		
Normal human plasma ‡.....	..	735 ± 216	225 ± 137	232 ± 62	82 ± 17	181 ± 71		
Fluid I**.....	..	...	814	None	..	274		
Fluid II.....	..	...	603	None	..	128		
Fluid III.....	..	...	1,501	None	..	360		
Fluid IV.....	..	...	100	None	..	23		
Plasma from fasting dog¶...	..	589	...	137				
Cervical lymph¶	..	305	...	56				

* If one fluid is left out, this becomes 202 ± 39.
† If one fluid is left out, this becomes 97 ± 24.
‡ If one fluid is left out, this becomes 41 ± 24.
# Chylous A was slightly to moderately milky; chylous B, very milky.
‡ Page and others.[7]
** Wallis and Schölberg.[4]
¶ Marble and others.[9]

opacity is evident. Owing to the fact that these fluids accumulated in the animals over long and irregular periods, it was impossible to compare their composition with that of the animal's blood during their formation.

It is evident from an examination of the figures in tables 6 and 7 that from the point of view of quantity the "neutral fat" constitutes the most important element in the lipids of our fluids. It averages from 70 to 87 per cent of the total lipids, and fluctuations in the total lipids are reflected accurately by fluctuations in neutral fat, as evidenced by the high coefficient of correlation in table 8. These figures support the assumption that our milky fluids are true chylous fluids.

The amount of cholesterol is always much smaller than the quantity of natural fat and varies with less regularity. It is interesting that the free cholesterol follows the total lipid much closer than does the bound cholesterol, as indicated by the figures in table 8. This may indicate that the source of the neutral fat fraction is also the main source of the free cholesterol, while bound cholesterol either finds its way into the fluids from some other source or some of it disappears from the fluid by reabsorption, decomposition or combination.

Inspection of the figures in table 7 reveals that the concentration of every lipid component except neutral fat was lower in the clear fluid than in normal blood plasma. Although the figures given in our table are for normal human plasma, we have a few for dog plasma which are of the same magnitude. Schally [8] reported figures for three "pseudochylous" fluids together with analyses of blood specimens from the same patients, and his values likewise were lower than those for the blood. On the other hand, our milky fluids were, as would be expected,

TABLE 8.—*Correlation Coefficients Between Total Lipids and Lipid Constituents*

Neutral fat	0.9688 ± 0.0108
Total cholesterol	0.6996 ± 0.0608
Free cholesterol	0.7073 ± 0.0893
Esterified cholesterol	0.5941 ± 0.0803
Total lipid nitrogen	0.4236 ± 0.0978
Amino lipid nitrogen	0.8610 ± 0.0281
Phosphatide	0.7270 ± 0.0803

all much richer than normal, nonlipemic plasma in total lipids and hence neutral fats, since this component exceeds the others. Nevertheless, although the very milky fluids were usually richer than plasma in phosphatide, the opposite is true for the less milky ones. Both types of fluids contained less cholesterol. Apparently the phosphatides increase more in proportion to the neutral fats than does the cholesterol.

We have been unable to find in the literature figures on the composition of lymph to afford any adequate comparison with our fluids. Marble, Field, Drinker and Smith [9] reported the total cholesterol and total lipid values for cervical lymph and blood plasma from dogs, which are included in our table and are of the same order of magnitude as the figures for our clear fluids. Their experiments indicated that blood capillaries are less permeable to cholesterol than to other lipids. The low values for our clear fluids indicate that other membranes have this property. They were unable to raise the cholesterol content of lymph by injecting cholesterol-containing fluids into the blood stream.

8. Schally, A.: Deutsches Arch. f. klin. Med. **174**:199, 1932.

9. Marble, A.; Field, M. E.; Drinker, C. K., and Smith, R. M.: Am. J. Physiol. **109**:467, 1934.

Bruger[10] found low values for total cholesterol in a series of fluids of various types, as did Man and Peters,[11] who also found no relation between serum and fluid cholesterol. Frölicher and Süllmann[12] found the ratio of free to esterified cholesterol to be unity in intestinal lymph except after fat feeding, when the proportion of free cholesterol was increased. There is no constancy about this ratio in our fluids, the free cholesterol being sometimes greater and sometimes less than the esterified cholesterol. The increase in cholesterol which took place when our fluids became milky, as well as the rather wide variation in successive withdrawals, can be explained on the assumption previously stated that the fluids were milky because of ruptures of lymphatics, which allowed the escape of chyle.

CHARACTER OF THE NITROGEN AND PHOSPHORUS COMPOUNDS IN FLUID LIPIDS

This subject has been thoroughly discussed in relation to the lipids of blood plasma in the various papers from the hospital of the Rockefeller Institute.[13] If the nitrogen were all in the form of lecithin or cephalin, the atomic nitrogen-phosphorus ratio should be 1, with the aminonitrogen-phosphorus ratio falling below 1 in proportion to the amount of lecithin in the mixture. In normal plasma the ratios found were much higher than unity, and in nephritic plasma, still higher than in normal plasma. The explanation offered was that nonphosphatide nitrogenous substances were extracted with the lipids. A similar situation seems to exist in fluids. There is a relatively low correlation between the total nitrogen and the total lipid, and the total nitrogen-phosphorus ratios are irregular.

The relationships for nitrogen are somewhat analogous to those for cholesterol in that the aminonitrogen shows a much higher correlation with the changes in total lipid than does the total nitrogen. There is an excellent parallelism between the aminonitrogen and total lipid, indicating that the aminonitrogen compounds are lipid in character, coming perhaps from the same sources as the neutral fats. The same may be said of the phosphorus compounds. As in blood plasma, the aminonitrogen-phosphorus ratios are usually well above unity, so that lecithin and cephalin are not the only constituents of this fraction. That aminonitrogen and phosphatides tend to rise and fall together is apparent from the similarity of the values for their correlation coefficients with neutral fats, their mean values and the correlation index of their own variation, which is 0.7496 ± 0.0522.

10. Bruger, M.: Am. J. Clin. Path. **5**:384, 1935.
11. Man, E. B., and Peters, J. P.: J. Clin. Investigation **12**:1031, 1933.
12. Frölicher, E., and Süllmann, H.: Biochem. Ztschr. **274**:21, 1934.
13. Kirk and others.[8] Page and others.[7]

It seems, then, that the lipid aminonitrogen and lipid phosphorus are mostly actually such but that, as in plasma, nonlipid, nonaminonitrogenous compounds contaminate the lipid extracts of our fluids to give variable values for total nitrogen.

We have carefully compared the figures in tables 5 and 6 in an attempt to discover some relation between cell count and chemical composition, but to no avail.

SUMMARY

Ligation of the superior vena cava has been found to produce milky fluids which are extremely rich in lymphocytes but which also contain considerable numbers of neutrophils.

In the circulating blood of animals the total number of lymphocytes and the total number of eosinophils drop markedly following ligation of the superior vena cava. The obstruction of lymphatic drainage is assumed to be of sufficient importance to account for the decrease in lymphocytes. No explanation so far has been obtained for the change in the circulating eosinophils.

Milky fluids produced by ligation of the superior vena cava in dogs are true chylous effusions, the opacity of which is due to free fat.

The lipids estimated as "neutral fat" in the method of analysis used comprise about 80 per cent of the total lipids. The composition of this fraction is such that the total lipid can be computed from it by multiplying the neutral fat values by 1.3. The quantities of free cholesterol are small but show a tendency to form a constant fraction of the total lipid. The esterified cholesterol, on the other hand, is less inclined to vary with the neutral fat. The total nitrogen values probably include nitrogen from nonlipid compounds. The aminonitrogen is, on the other hand, mostly due to true lipids, and it tends to vary with the neutral fat. The phosphatide fraction contains substances which are neither lecithin nor cephalin. These, too, tend to vary with the neutral fats. There is a sufficiently strong correlation between the phosphorus and aminonitrogen to indicate that they, to a large extent, rise and fall together.

ANATOMIC CHANGES PRODUCED BY THYROID FEEDING AND BY INJECTION OF 3,5-DIIODOTYROSINE

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Most experiments designed to determine the effects of thyroxin and related substances on the animal body have been in the nature of acute, short time ones, with observations on the basal metabolism and associated physiologic changes chiefly. A few, such as the earlier work of Goodpasture [1] and the more recent work of Menne and his associates,[2] and Rake and McEachern,[3] were concerned with the possible anatomic changes in the heart. These were also comparatively acute experiments, those of Goodpasture covering twenty-six days (the longest period), Menne's twenty-three days and Rake's from two to eighty-three days. In all these investigations, myocardial degeneration was produced by feeding thyroid substance or by injecting thyroxin U. S. P. in practically all animals studied.

Clinical and pathologic reports of cardiac lesions in hyperthyroidism are abundant, but there has been a tendency to dismiss these as unimportant, not specific, not permanent, or incidental.[4] Six cases of this description appear in the autopsy records of this department, covering a period of eight years; i. e., cases of death from heart failure without the excessive hyperthermia of critical thyrotoxicosis and without significant incidental findings. Two of the patients were operated on in desperate attempts at treatment, with use of gas (nitrous oxide) and oxygen anesthesia. These were all cases of chronic thyroid hyperfunction of a low grade. The following is a good example:

N. A. S., a woman of 49 years, had had thyroid enlargement for nineteen years and had suffered from excessive nervousness, loss of weight (48 pounds [21.8 Kg.]) and palpitation of the heart for seven months. Exophthalmos was not present.

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The investigation was supported by the Christine Breon Fund for Medical Research.

1. Goodpasture, E. W.: J. Exper. Med. **34**:407, 1921.

2. Menne, F. R.; Keane, R. H.; Henry, R. T., and Jones, N. W.: Am. Heart J. **8**:75, 1932. Menne, F. R.; Jones, O. N., and Jones, N. W.: Arch. Path. **17**:332, 1934.

3. Rake, G., and McEachern, D.: Am. Heart J. **8**:19, 1932.

4. See the symposium on this subject published in the American Heart Journal (**8**:1, 1932).

The pulse rate was 130 to 140; the blood pressure, 150 systolic and 84 diastolic; the basal metabolic rate, + 51. The electrocardiogram showed definite myocardial damage: auricular fibrillation, depressed T intervals and inverted T_2 and T_3. There were ventricular extrasystoles. The clinical diagnosis was: toxic nodular goiter and thyrotoxic heart disease. She was operated on and 142 Gm. of thyroid tissue removed. The pathologic diagnosis was: nodular goiter with hyperplasia. The patient continued to suffer from fibrillation and died three days after operation, with congestive heart failure. Autopsy showed enlargement of the heart (weight, 380 Gm.; left ventricle, 2 cm.), hydrothorax, hydropericardium and passive congestion of the lungs and liver. The heart, histologically, presented a good example of so-called chronic fibrous myocarditis, with more recent acute degeneration in some areas. There was no terminal infection.

Such a sequence seems clearly to have been due to one cause only, chronic thyroid intoxication, and it is unnecessary to postulate other incidental, accessory or concomitant factors to explain the clinical course or the pathologic appearances at autopsy. A condition similar to it ought to be relatively easy to produce in an experimental animal.

During the long search for explanations for the varied and variable conditions associated with a diseased thyroid gland, attempts have been made to determine the behavior of the iodine-containing and noniodine-containing substances chemically related to or entering into the formation of thyroxin. These experiments were a natural development as different chemicals were isolated from thyroglobulin but were stimulated also by the fact that late effects of thyroid disease (as in "toxic adenoma") were found to be different from those found in acute or primary thyroid hyperplasia with exophthalmos. This late manifestation was called dysthyroidism (attributed to McCarrison in England and to Plummer in this country), and an "altered" or incompletely formed secretion was imagined as the possible cause. Among such substances now isolated are tyrosine, 3,5-diiodotyrosine, thyronine and 3,5-diiodothyronine, and thyroxin itself. Reports of the effects of these chemicals on experimental animals are now appearing, with some apparently contradictory results. For instance, Canzanelli and Rapport [5] reported that tyrosine itself will raise the basal metabolism of dogs by 10 per cent; diiodotyrosine, more; diiodothyronine, still more, and thyroxin, the most. On the other hand, l-tyrosine inhibited the action of thyroxin on such factors as basal metabolism and body weight in treated rats (Abelin [6]) and reduced the basal metabolic rate, on the average, 22.5 per cent in patients suffering from hyperthyroidism (Delcourt-Bernard [7]). In Germany, particularly, diiodotyrosine has been used in place of iodine in preparation for operation, and Buresch [8] has reported complete cures

5. Canzanelli, A., and Rapport, D.: Am. J. Physiol. **103**:279, 1933.
6. Abelin, I.: Klin. Wchnschr. **14**:1777, 1935.
7. Delcourt-Bernard, E.: Compt. rend. Soc. de biol. **122**:820, 1936.
8. Buresch, A.: München. med. Wchnschr. **81**:320, 1934.

of hyperthyroidism in some cases. All experiments with diiodotyrosine and related substances have also been short time, rather acute investigations. Whether prolonged administration of any of these chemicals produces noticeable anatomic changes has not been determined.

EXPERIMENTAL OBSERVATIONS

Somewhat over a year ago, two groups of rabbits were started on a long time experiment with thyroid substance and 3,5-diiodotyrosine. Ten rabbits received 60 mg.[9] per kilogram of desiccated thyroid U. S. P. by mouth each day, six days a week, for upward of ten months. Twelve rabbits received 2 mg. per kilogram of diiodotyrosine the same number of times for eight months of the ten month period. It is the purpose of this paper to report the results of these simple experiments.

The accompanying graph (fig. 1) shows the rate of growth of the treated animals. These were young, immature rabbits, and the average weight of each group

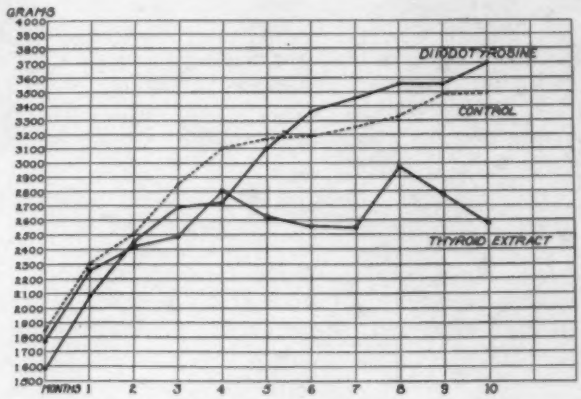

Fig. 1.—Record of weights, from month to month, of rabbits fed thyroid substance or given injections of diiodotyrosine.

is indicated (there were six untreated controls selected to approximate in weight and age the treated groups). This is significant only in that it shows a definite effect of the thyroid and a doubtful effect of the diiodotyrosine on the body weight. Between the fourth and sixth months, injections were not given to the diiodotyrosine-treated rabbits, and during that time there was a noticeable increase in the rate of growth of that group. This may be an expression, therefore, of some growth-retarding effect of that substance. However, the end-result was an excess in weight over all controls, associated, as will be seen, with a subnormal thyroid gland.

Other objective findings during the course of the experiments were an increased heart rate (to from 270 to 290 beats per minute) in the thyroid-treated group and a suggestion of bilateral exophthalmos (not measured) in three thyroid-treated rabbits. Electrocardiograms were made after eight months of treatment, and these showed "myocardial damage," but so did some of the controls. Since I sought anatomic changes, the usual physiologic and chemical tests were omitted.

9. Approximately 0.4 mg. of thyroxin U. S. P.

Thyroid-Treated Rabbits.—Heart: The hearts of seven animals, which were allowed to live the entire ten months of the experiment, averaged 7.8 Gm. in weight. The smallest weighed 5.4 Gm.; the largest, 9.5 Gm. All showed changes which varied from simple hyaline degeneration to acute patchy necrosis. Three contained small scars, in addition, and all of them contained large or small patches of cells growing from the stimulated interfascicular tissues. These effects, in general, were not striking and were injuries from which the myocardium should have recovered, with no sign left save comparatively small, insignificant scars, after treatment was stopped. Three, however, contained many areas of acute necrosis, as shown by characteristic fading of muscle cells, these becoming frayed-out patches of fibrillary nonstaining débris, such as may be seen associated with acute intoxication in diphtheria and other severe poisonings (fig. 2 *A*). In one rabbit this acute myocardial degeneration was so severe as to cause death. This animal was started on 60 mg. of thyroid substance a day, and this was gradually raised to 180 mg. It had been apparently normal at the start, but, along with several others, it had to be nursed along carefully when loss in weight or lack of growth became too pronounced. On two occasions treatment was stopped for a week. The rabbit died after three and a third months with dilatation of the heart, marked edema and congestion of the lungs and liver, without other lesions or contributory cause. This death is regarded as one from myocardial failure only. The heart weighed 6.3 Gm. and was the seat of severe diffuse myocardial degeneration (fig. 2 *B*). The liver contained areas of focal necrosis separable from and unassociated with the severe central congestion due to stagnant circulation (fig. 3 A.)

Endocrine Organs: There were seven female and three male rabbits in the thyroid-treated group. In six of the seven females, the uterus, tubes and ovaries became atrophied. This was both a gross and a microscopic change. Histologically, the endometrium consisted of one layer of cells on a comparatively smooth, uncorrugated subepithelial layer, the usual papilliform structure having disappeared. These cells showed varying degrees of mucoid degeneration. There were some sections in which all the epithelial cells were markedly distended with mucus. The ovaries of these animals were inactive and fibrotic but not entirely senile. All contained a few small unripe follicles but no lutein cells. As a contrast, the seventh female, treated the full ten months also, had a large succulent uterus and large ovaries. In this the changes were those of pseudopregnancy and the ovaries were converted almost completely into lutein bodies, with only a few small follicles containing ova lying in this luteinic stroma.

The testes of two of the three males became markedly atrophic; those of the third were small but remained active. Sections of the atrophic testes showed complete cessation of spermatogenesis and a shrunken condition of the tubules, which were small and lined usually with only one layer of cells.

The thyroid gland in every rabbit became inactive. It is practically impossible to weigh the gland, but compared with the controls these glands were larger and contained much more colloid, and the alveolar lining cells were flattened out to an inconspicuous thin line, the whole approaching the appearance of colloid goiter (fig. 3 *B*). This was most pronounced in the pseudopregnant rabbit. The weight of the thymus varied from only 0.7 to 3.5 Gm. Histologically, both the medulla and the cortex stood out plainly, with abundant cells and no involution changes but, as usual, a distinctive change from the normal could not be recognized. Observations on the adrenals were somewhat more satisfactory. All were probably larger than usual, taking into account the uncertainty as to what a normal rabbit adrenal ought to weigh, and the fact that these were all emaciated, practically fat-free animals. Histologically, the cortex was one wide zone of cells rich

in lipoid, with none of the smaller, more deeply staining cells usually found beneath the capsule, which in other animals form a glomerular zone. One contained a cortical adenoma 5 mm. in diameter, and in one there were small collections of accessory cortical cells in the surrounding fat. Medullary tissue was present in the usual amount.

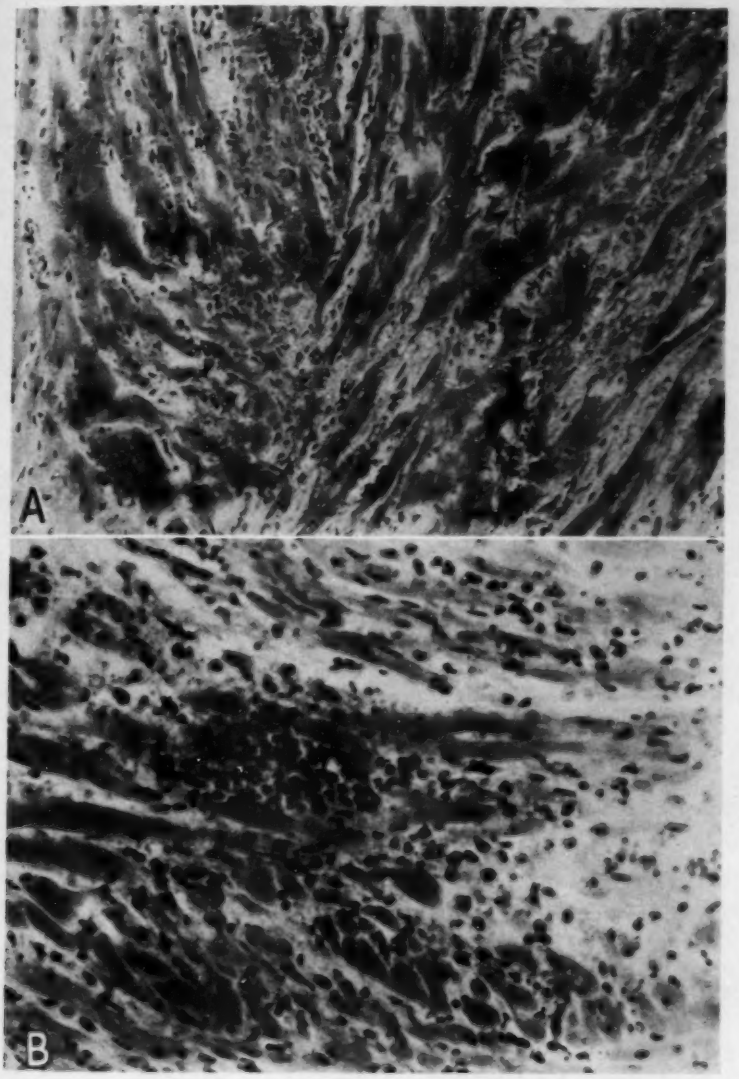

Fig. 2.—*A*, hyaline degeneration and necrosis in heart muscle after ten months' treatment. The specimen was taken from a weakened, emaciated rabbit which was killed. *B*, acute myocardial necrosis in a rabbit which died after three and a third months of treatment, of heart failure.

The pituitary glands were stained with Mallory's connective tissue stain as well as by the ordinary hematoxylin and eosin method. The gland from the pseudopregnant rabbit showed a majority of cells staining pink with hematoxylin and

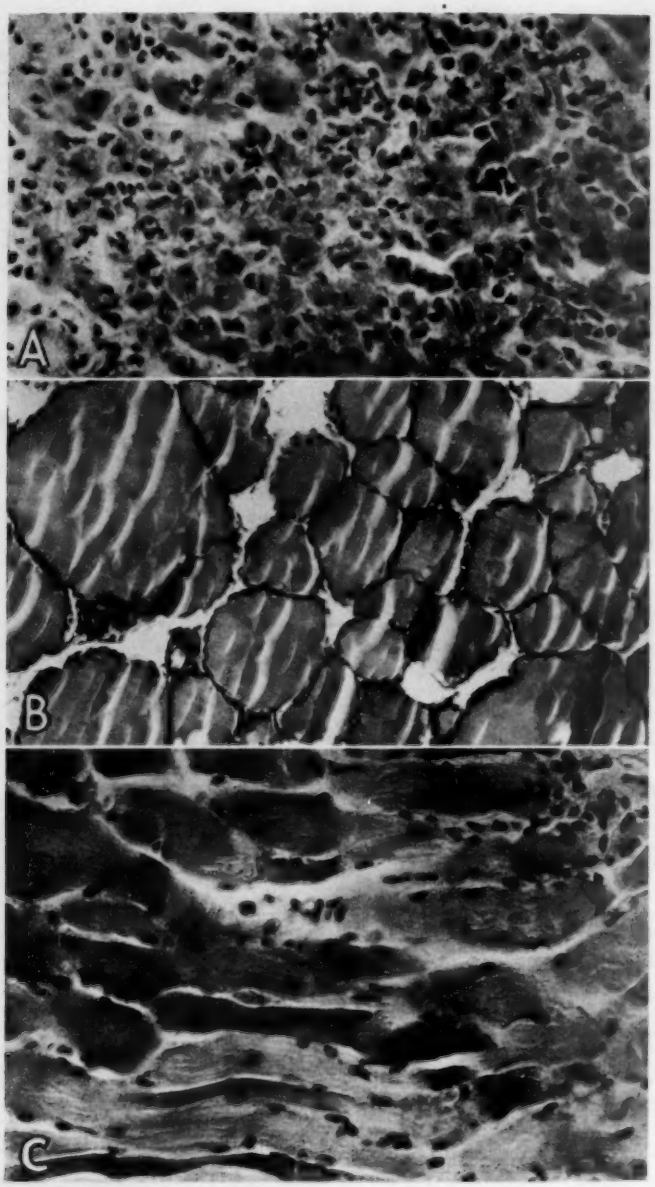

Fig. 3.—*A*, focal necrosis of the liver in thyroid-treated rabbit. *B*, quiescent, colloid type of thyroid after prolonged thyroid feeding. *C*, swelling, hyaline degeneration and necrosis in an eye muscle of a rabbit after prolonged thyroid intoxication.

eosin, which still were not the usual eosinophilic granular cells of the normal gland. They resembled the altered chromophobe cells which Erdheim described in the hyperplasia of pregnancy and which are now called variously chief cells, castration cells or pregnancy cells. The other glands varied from essentially normal (even in the two animals with testicular atrophy) to definitely abnormal glands in two females with atrophy of the generative organs. The last were made up of chromophobe cells mostly, with few eosinophils and fewer basophils, all of them small with scanty cytoplasm, and what appeared to be some fibrosis. This appearance, however, was due to the shrinkage in size and possibly numbers of cells, resulting in a condensation of the stroma, which thus became more prominent and conspicuous. In the less affected glands, the changes amounted to a simple reduction in the relative number of true eosinophils.

Other Tissues: The liver, spleen, breasts, skeletal muscle (pectoralis major, muscles around the larynx) and the extrinsic muscles of the eyes were examined. The livers, except the one noted, were not affected (weight from 72 to 175 Gm.). The spleen, likewise, showed no consistent change except that in two animals it could be said to be large. The breasts of two females with atrophic ovaries were definitely enlarged owing to a cystic hyperplasia affecting mostly the ducts, but there is no possible correlation of these changes with the other changes, as most breasts were atrophied. In two animals there was questionable degeneration of skeletal muscles, but three showed indubitable degenerative changes in eye muscles. These consisted of swelling, loss of striations and patchy necrosis with round cell proliferation approaching closely the picture found in chronic exophthalmic thyrotoxicosis (fig. 3*C*).

Diiodotyrosine-Treated Rabbits.—Of six male and six female rabbits started on daily injections of 2 mg. per kilogram of 3,5-diiodotyrosine, two males died after nine months of intercurrent infections. The remainder were all in good health when killed at the end of ten months. The dose was arbitrary and perhaps small, but it represented considerably more iodine than could be given as thyroxin. Goodpasture gave only 1 mg. of thyroxin to rabbits every second or third day to produce heart changes. Buresch gave from 50 to 100 mg. of diiodotyrosine to women with hyperthyroidism to produce the pronounced effects described. Delcourt-Bernard gave only 1 mg. per kilogram per day for seven days to man to produce the same effect. Canzanelli and Rapport injected 200 mg. into a dog, seeking an immediate and measurable effect. To duplicate a possible chronic thyroid dysfunction, relatively small doses over a long period of time seemed indicated.

As in the thyroid-treated animals, all organs which might conceivably be affected by this treatment were examined grossly and histologically. The results on the heart, liver, spleen, skeletal muscle, eye muscles and thymus were essentially negative. The thyroid gland in every animal could be described as quiet, relatively inactive, with perhaps some alveoli larger and the epithelium more flattened than usual. There were, however, definite and somewhat surprising changes in the ovaries, testes and accessory genital structures.

All six females had the marked ovarian and uterine enlargement described in rabbit 12 of the thyroid-treated group, with widened adrenal cortices but without marked changes in the pituitary glands. All the ovaries in this group were converted into lutein bodies, with no ripe follicles and few unripe follicles. The uterine mucosa was papillary, very cellular, and could be said to be definitely hyperplastic. In the males, the seminal vesicles were atrophied to the extent that they were found with some difficulty, and on histologic examination the epithelium and glandular tissue of both vesicles and prostate were less complex than normal.

The testes were active in that there were the several "layers" of lining cells, but spermatozoa and mitotic figures were few. In other words, there was a slowing down in activity to a point approaching but not actually reaching definite atrophy.[10] In these, also, the adrenal cortical tissue was abundant and full of lipoid substance.

COMMENT

It has been shown again that an excess of thyroid secretion in the animal body, of either endogenous or exogenous source, will produce definite anatomic changes in the heart and that these may be severe enough to contribute materially to final functional exhaustion. They are changes, however, from which the heart should recover completely after removal of the cause of the intoxication, and the physiologic and chemical changes induced are probably the more important. However, since death from functional change is just as definite and lasting as that from anatomic change, the seriousness of prolonged thyroid intoxication is not hereby minimized, but emphasized. There seems to be no question but that, both clinically and experimentally, there is such a condition as pure, uncomplicated thyroid heart failure and that this needs more than perfunctory recognition in order to prevent possible fatalities.

Muscle changes were looked for because of the definite degenerations which occur in the eye muscles in chronic exophthalmic goiter. That they were found in three animals confirms in experimental animals the previous clinical and pathologic observations to the same effect. The changes in the skeletal muscles were not of the type of severity found in well advanced myasthenia gravis (not of the type, probably because they were not severe enough). Theoretically, such changes ought to be produced by long-continued thyroid intoxication of a low grade. Bodansky and Pilcher [11] have produced degeneration of both cardiac and skeletal muscle and, by chemical analysis, loss of glycogen and creatine by injecting into rats 1 mg. of thyroxin U. S. P. daily for twenty-three days. Such findings, along with clinical and pathologic evidence pointing to thyroid (as well as thymus) dysfunction in myasthenia gravis, lead to the thought that a similar condition may be eventually reproduced in experimental animals.

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10. Statements made here regarding slight changes in organs are not meant to be equivocal. They do indicate, however, the difficulty in estimating the degree of activity of a gland by its histologic appearance. All organs and tissues of the endocrine system and of the reproductive system undergo such astonishing changes which cannot be correlated that definite assertions as to whether they are normal, hyperplastic or hypoplastic under the conditions of the experiment cannot be made. There have been too many positive statements of positive effects in experiments in which all the factors which may alter the histologic appearance of the endocrine glands have not been controlled.

11. Bodansky, M., and Pilcher, J. F.: Proc. Soc. Exper. Biol. & Med. **35**:607, 1937.

Marked necrosis of the liver in thyroid disease has been described by Kerr and Rusk.[12] Since that report, there has been observed in the autopsy service of this department a case of diffuse gross acute degeneration of the liver without other explanation than the thyrotoxicosis from which the patient died. The appearance of focal necrosis in one animal, as recorded here, indicates again that necrosis of the liver may be produced by long-continued thyroid intoxication alone.

Thyroid feeding also produced changes in endocrine glands and in the reproductive organs which have not hitherto been described. The change of the thyroid gland into a less active gland with more colloid and smaller epithelial cells would be expected when more than sufficient thyroid secretion is supplied from other sources for a considerable period of time.

Ovarian, testicular and uterine changes occurred also because of the length of time the animals were treated, and the apparent contradictory effects must be due to variation in the time required to produce certain specific effects. Such variations occurred in a group of animals treated with the urine of pregnant women for several months.[13] Some animals of the same group had hyperplastic uteri or prostates, according to sex, and others had atrophy of corresponding organs. A reexamination of that group indicates that a stage of hyperplasia is followed in time by atrophy, and that the time, within rather broad limits, is peculiar to each animal. Mazer, Israel and Alpers[14] treated rabbits with dihydrotheelin benzoate (the benzoate of 3, 7-dihydroxy-1, 3, 5-estratriene), 10,000 rat units daily for seven days, and produced enormous growth of the uterus and luteinization of the ovaries in that period; in other rabbits treated similarly for twenty-one days, both the uterus and the ovaries became atrophic. A like sequence must be postulated to explain the different changes in thyroid-treated rabbits.

The changes in the pituitary can be correlated more closely with the changes in the uterus and ovaries than with any other change: hyperplasia of altered chromophobe cells in the pseudopregnant rabbit and relative atrophy in those with atrophic genitalia. These experimental changes, again, are reproductions of similar conditions found in women: the well known amenorrheas in hyperthyroidism and the metrorrhagia and menorrhagia of hypothyroidism. They may explain, also, why such clinical findings are so irregular and not constant.

Evidence is accumulating that not all or at least not the most pronounced effect of thyroid secretion resides in thyroxin alone. Means,

12. Kerr, W. J., and Rusk, G. Y.: M. Clin. North America **6**:445, 1922.
13. Unpublished experiments on rats, rabbits, guinea-pigs and dogs.
14. Mazer, C.; Israel, S. L., and Alpers, B. J.: Endocrinology **20**:753, 1936.

Lerman and Salter [15] found that thyroxin plus the other organic iodine-containing fraction of iodothyroglobulin was more active than thyroxin alone. But there is not enough evidence yet that the other active substance is diiodotyrosine. The effect of this substance on the uterus and ovaries is associated with a subnormal thyroid. Ovulation and pseudopregnancy can be induced in a rabbit by various manipulations, but there was no evidence that ovarian follicles had ruptured in these animals. The lutein cells had not replaced corpora hæmorrhagica, but there had been complete replacement of the ovary, outside of a few small follicles, with lutein cells. There were no ripe follicles, few hemorrhagic bodies (in unruptured follicles) and very few primordial follicles. The condition of the pituitary glands in these animals was not characteristic of genuine pregnancy.

SUMMARY

Rabbits which had been fed 60 mg. per kilogram of thyroid substance daily for ten months became extremely emaciated, showed enlargement of the heart with focal areas of necrosis in the myocardium, and one animal died of myocardial failure alone. There was also definite degeneration of the eye muscles in three rabbits similar to that found in chronic exophthalmic goiter. The thyroid glands of all were large and showed the histologic picture of an inactive colloid storage gland. Other endocrine changes included atrophy of the ovaries and uteri of six of seven females and atrophy of the seminal vesicles, prostate and testes in three males. One female had a large uterus and large ovaries replaced by lutein cells. The pituitary of this animal showed chromophobe hyperplasia; these glands in the others showed relative atrophy. The adrenals were large and active in all of them.

Rabbits which had been given injections of diiodotyrosine for eight months in a period of ten months showed no muscle changes, but all females had enlargement of the ovaries and uteri similar to but not identical with pseudopregnancy. In these also the thyroid became relatively inactive; the pituitary remained essentially normal.

15. Means, J. H.; Lerman, J., and Salter, W. T.: J. Clin. Investigation **12**: 683, 1933.

WAVE MECHANICS OF PROTOPLASMIC ACTION

XI. EXPERIMENTAL HISTOLOGY OF NERVE FIBERS

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The physical nature of either the normal or the abnormal neural impulse and the means by which the impulse is conducted in the axis-cylinder of a nerve fiber are unknown. After Remak [1] discovered neurofibrils in the living nerve fibers of the crayfish, Schultze,[2] Apathy [3] and Bethe [4] presented the hypothesis that the neurofibrils were the specific wirelike conductors of the nerve impulse. What went up or down the neurofibrils to constitute a nerve impulse was unknown.

The prevalent opinion expressed in most textbooks of histology is that the neurofibrils are the specific electric-like core conductors of the neural impulse. Bozler [5] and de Renyi [6] found neurofibrils in living nerve fibers of invertebrates. Parker [7] stated that the neurofibrils control the metabolic trophic activities of the neuron, whereas Verworn,[8] von Lenhossek,[9] Koltzoff,[10] Goldschmidt [11] and von Szuts [12] held that the neurofibrils in the axon are supporting structures for the semifluid neuroplasm.

The theory that the conduction medium of the nerve impulse is a liquid is supported by the observations of Jenkins and Carlson,[13] Carl-

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1. Remak, R.: Arch. f. Anat., Physiol. u. wissensch. Med. **2**:197, 1843.
2. Schultze, M., in Stricker, S.: Handbuch der Lehre von den Geweben, Leipzig, W. Engelmann, 1871, p. 108.
3. Apathy, S.: Mitt. a. d. zool. Station zu Neapel **12**:495, 1897.
4. Bethe, A.: Zentralbl. f. Physiol. **21**:404, 1907.
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6. de Renyi, G. S.: J. Comp. Neurol. **47**:405, 1929.
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11. Goldschmidt, R., in Festschrift zum 60. Geburstage Richard Hertwig, Jena, G. Fischer, 1910, vol. 2, p. 253.
12. von Szuts, A.: Anat. Anz. **47**:199, 1914.
13. Jenkins, O. P., and Carlson, A. J.: J. Comp. Neurol. **14**:85, 1904.

son,[14] Meek and Leaper,[15] Lapicque and Legendre,[16] Erlanger,[17] Erlanger and Gasser,[18] Gasser and Erlanger,[19] Young [20] and Gerard.[21]

Certain physiologists, Lillie [22] and Adrian,[23] in order to explain the conduction of the nerve impulse, support the hypothesis that the resting nerve fiber is enclosed by a polarized membrane. This is assumed to break down locally under the action of a stimulus establishing an action potential or gradient difference between the active and inactive regions.

Because a measurable difference of electrical potential has been found to accompany the local excitation process it is assumed that this action potential serves as a stimulus to the resting region adjoining the active area. The electric core conductor theory is based on the assumption that the nerve impulse is transmitted through a nerve in a manner analogous to the transmission of the chemical change exhibited by a system consisting of a core surrounded by a membrane, sheath or layer of electrolyte solution. The histologic structural response of the axis-cylinder in a nerve to any stimulus, however, is unknown.

Harrison [24] gave experimental proof that nerve fibers grow out of the cell bodies, at an early stage of development, like the protrusion of a pseudopod from an ameba. The nerve fiber begins as an outgrowth of hyaline protoplasm from the nerve cell. The mechanism of this protrusion of protoplasm in unknown. The experiment suggests that the solution of the problem of conduction and action in ameboid movement may throw light on nerve action. The solution, however, of the problem of nerve action may be the means of explaining ameboid motion. Kappers [25] designated the directed growth of nerves as neurobiotaxis.

Bremer and Rylant [26] found that the chronaxia, the time factor of Lapicque in excitation, is decreased with increase of excitability when strychnine is applied locally to the peripheral sensory and motor nerve

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fibers in mammals. Dusser de Barenne [27] gave evidence that the strong "typical strychnine tetani occur only when the cell bodies (*perikarya*) in the dorsal horns of the spinal cord are poisoned simultaneously with the cell bodies of the motor cells in the ventral gray matter of the spinal cord."

The exploration, experiments and final detection of the evidence for longitudinal pressure waves conducted through a microcapillary tube containing a liquid as the biophysical basis of the nerve impulse are logical consequences of earlier studies (Carey [28]) in the dynamics of histogenesis and the wave mechanics of muscle function. If the motor nerve-ending is an oscillating radiator from which microcapillary pressure waves emanate in cross-striated muscle, there should be an intermittent pressure activator of the terminal nerve vibrator. The microcapillary pressure pulses, represented as periodic wavelike structures in the liquid axis-cylinders of nerve fibers, are assumed to be the activators of the vibratory motor nerve-endings. These active nerve-endings radiate internal pressure waves in muscle. These waves are structurally expressed as cross-striations. Anastomotic muscle fibers like those in the heart and branched fibers within certain skeletal muscles have constructive and destructive interference patterns of these muscle pressure waves histologically expressed as intercalated disks. The multiplication of muscle pressure waves, with the reversible replacement of long by short waves, underlies the shuttle-like shifting and splitting of the cross-striations in the normal contraction and abnormal rigors of muscle.

Fortunately the histologic results of this study of active and inactive nerve fibers may be seen at a glance in the photomicrographic records, which need little morphologic description. There is only one means of realizing the great variability of the pressure waves conducted through nerves and that is by studying an adequate number of photomicrographs. Even then the observer must visualize the reversible motions of the longitudinal progression of pressure waves, namely, the alternate centrifugal expansions and centripetal constrictions of a particular place of the axis-cylinder under observation in the photograph. There is no other way to convey to the mind of the reader the changeable character of the spectra and of the different phases during the conduction of the nerve pressure waves than by an apparent abundance of selected photomicrographs. With this evidence at hand, others may come to their own conclusions, and any hypothesis advanced, except as a motive for investigation, becomes relatively immaterial.

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The purpose of this paper is to present histologic evidence, in photomicrographs, of wavelike structural deformations in most of the axis-cylinders of the brachialis and sciatic nerve fibers of the frog (Rana pipiens) and the cat killed by strychnine. In the abnormal quiescent state of an isolated nerve these histologic signs of nerve action are absent. During deep anesthesia these wavelike structures are diminished in number and in size. In many of the nerve fibers there are no deformations of the axis-cylinders under prolonged deep narcosis. Physiologists have demonstrated the electrical, metabolic and thermal signs of nervous activity. The anatomist, however, has not demonstrated the histologic appearances of the active and of the inactive nerve fiber. There is a correlation, heretofore undetected, between normal and abnormal nerve function and nerve structure, illustrated in this paper. In the differentially active nerve trunk, there is histologic variation in the number and size of the wavelike deformations in each axis-cylinder and in the number of axis-cylinders functionally involved in the conduction of the impulse. These histologic wave patterns of the axis-cylinders are quickly destroyed in nerve fibers rendered completely inactive by severance from their controlling cell bodies and functional end-organs.

METHOD

The method used is as follows: Twelve frogs (Rana pipiens) and six cats are killed with convulsive doses of strychnine. Two cats are killed quickly during the anesthetic excitation stage by ether. Three are each given 25 cc. of ethyl carbamate (urethane) by stomach tube and placed in the dark for four hours, then etherized for three hours and at the end of this period are killed by an overdose of ether. The nerves of these animals are studied for contrasting histologic differences between the initial excitation and the final deep, relaxed stages of narcosis. The nerves are prepared as follows: 1. The peripheral nerves are exposed, left in situ, fixed and stained simultaneously for six hours in equal parts of solution of formaldehyde U. S. P. in 20 per cent dilution and 0.5 per cent thionine. 2. The nerves are excised and fixed with terminal rubber bands on glass slides, which are placed in the formaldehyde-thionine solution for an additional forty-eight to seventy-two hours. 3. The nerves are washed in distilled water for ten minutes. 4. The nerve to be examined is placed in a porcelain cup with a perforated bottom. 5. The cup is suspended in the upper part of a glass container filled with dioxane. The lower part of the glass container supports a layer of calcium chloride, which combines with the free water displaced from the tissue. The nerve is retained in the pure dioxane for twenty-four hours. 6. It is transferred to pure paraffin for six hours and again to pure paraffin for another six hours. 7. After blocking, the nerve is cut in serial sections, 8 microns thick, and the sections are mounted on glass slides.

This formaldehyde-thionine-dioxane method is superior to any method in which either metallic impregnation of the nerves or staining with osmium tetroxide is used. The axis-cylinders are revealed in a clearer manner by the method of preparation outlined than by any other process of fixation and staining. The astringent and hardening actions of the xylene and alcohol used in other methods

prior to mounting the sections on glass slides are avoided. The delicate wavelike structures in the liquid axis-cylinders, with a high water content, are preserved by the formaldehyde-thionine-dioxane technic.

Opposed to the increase of nervous activity produced by strychnine is the decrease following complete transection of the brachialis and sciatic nerves in two places 1 inch (2.5 cm.) apart. In two of the animals the part of the nerve between the two transverse cuts is immediately removed; in six animals the cut nerves remain in their natural places for one, two, three, four, five and six hours, respectively, before being removed. The nerves on the left side are operated on, while those on the right, in three of the animals, are used as normal controls. The segment of the nerve between the transverse sections is sufficiently isolated from the cephalic and caudal stumps of the trunk by wide gaps to prevent ordinary nervous transmission through the experimental isolated segment. After the nerve segments are removed from three of these frogs, the animals are killed by strychnine. The inactive nerve segments are then contrasted histologically with their hyperactive fellows of the opposite side.

EXPERIMENTAL RESULTS

There are regular and irregular periodic wavelike structures in the axis-cylinders of the hyperactive nerve excised from an animal stimulated by a lethal dose of strychnine (figs. 1 and 2). Part of the left median nerve between the places of two transverse sections is inactive in the living frog for three hours (fig. 3). The transverse diameters of the large fibers of this inactive brachialis nerve are about one-half those of intact fibers of the corresponding type from the right median nerve when the animal was killed by strychnine. Clearly visible is the difference between the smooth, uniform structure of the retracted axis-cylinders of the inactive left median nerve and the wavelike structure of those of the right brachialis nerve, which is stimulated to hyperactivity by strychnine (contrast figs. 2 and 3).

During the excitation stage of ether anesthesia there are wavelike structures in the axis-cylinders of most of the nerve fibers in the sciatica nerve of the cat (fig. 5). After prolonged anesthesia, four hours with ethyl carbamate and then three hours with ether, the axis-cylinders have a relatively smooth, uniform contour (fig. 4).

The sciatic nerve from a cat killed with strychnine has, in certain places, abnormal bulgings of the axis-cylinders. These are visible in the photomicrograph as oblong dark dilatations, which stain red with thionine (figs. 6 and 8). There is an effect of cross-striation when the nerve trunk is observed microscopically in both low and high powers, because the histologic signs of nerve action become alined when the entire group of axis-cylinders is brought into functional synchronization by strychnine (figs. 6, 7, 8 and 9). This is structurally expressed in the axis-cylinders by a moniliform or beaded pattern of alternate dilatations and constrictions. If the dilatations of the axis-cylinder come in direct contact with the outer neurilemma, the myelin is squeezed out

PLATE I—EXPLANATION OF FIGURES 1, 2 AND 3

Figs. 1 and 2.—Photomicrographs (untouched) of a longitudinal section of the left brachialis nerve of a frog (Rana pipiens) killed with strychnine: fig. 1, × 175; fig. 2, × 720. The small figures *1, 2, 3, 4* and *5* indicate (*1*) an axon, (*2*) myelin, (*3*) the neurilemma, (*4*) a Schmidt-Lantermann band of temporary adhesion between the axon and the neurilemma, and (*5*) a node of Ranvier. There is a characteristic wavelike histologic pattern in the axon (axis-cylinder) of almost every one of the nerve fibers of a nerve trunk stimulated by a lethal convulsive dose of strychnine. When the nerve becomes hyperactive, there appears to be synchronism in the volley of waves fired through most of the axons. There appears to be a quantitative increase in the number of fibers with axonic wavy displacements during augmented action of the nerve. The histologic expression of increased nerve function is the appearance of alternate expansions and constrictions of the axons of most of the fibers in a nerve trunk. Since strychnine increases both the sensory and the motor activities, both types of fibers in the mixed median nerve have histologic changes. Experiments on the sciatic nerve confirm this.

Fig. 3.—Photomicrograph (untouched) of a longitudinal section of the right brachialis nerve excised from the same frog prior to the injection of strychnine; × 720. The small numbers *1, 2, 3* and *4* have the same indications as in figs. 1 and 2. The brachialis nerve was cut in two places ½ inch (1.27 cm.) apart. After the two transverse incisions were made, the nerve remained quiescent, in situ, for three hours before it was removed from the animal. Care was taken to prevent conduction through the isolated segment by making a wide gap between it and the proximal and distal stumps. The inactive brachialis nerve segment isolated from its terminal end-organs and motor and sensory cell bodies is completely devoid of wavelike deformations in any of its axons. The nerve fiber and its component structures are abnormally narrow compared with its fellow of the left side stimulated by a lethal subcutaneous dose of strychnine (figs. 1 and 2). The axon in the inactive nerve is narrow and has a smooth, uniform cylindric contour. There are different histologic nerve patterns correlated, therefore, with hyperactivity and inactivity of nerve function. Experiments on the sciatic nerve confirm this.

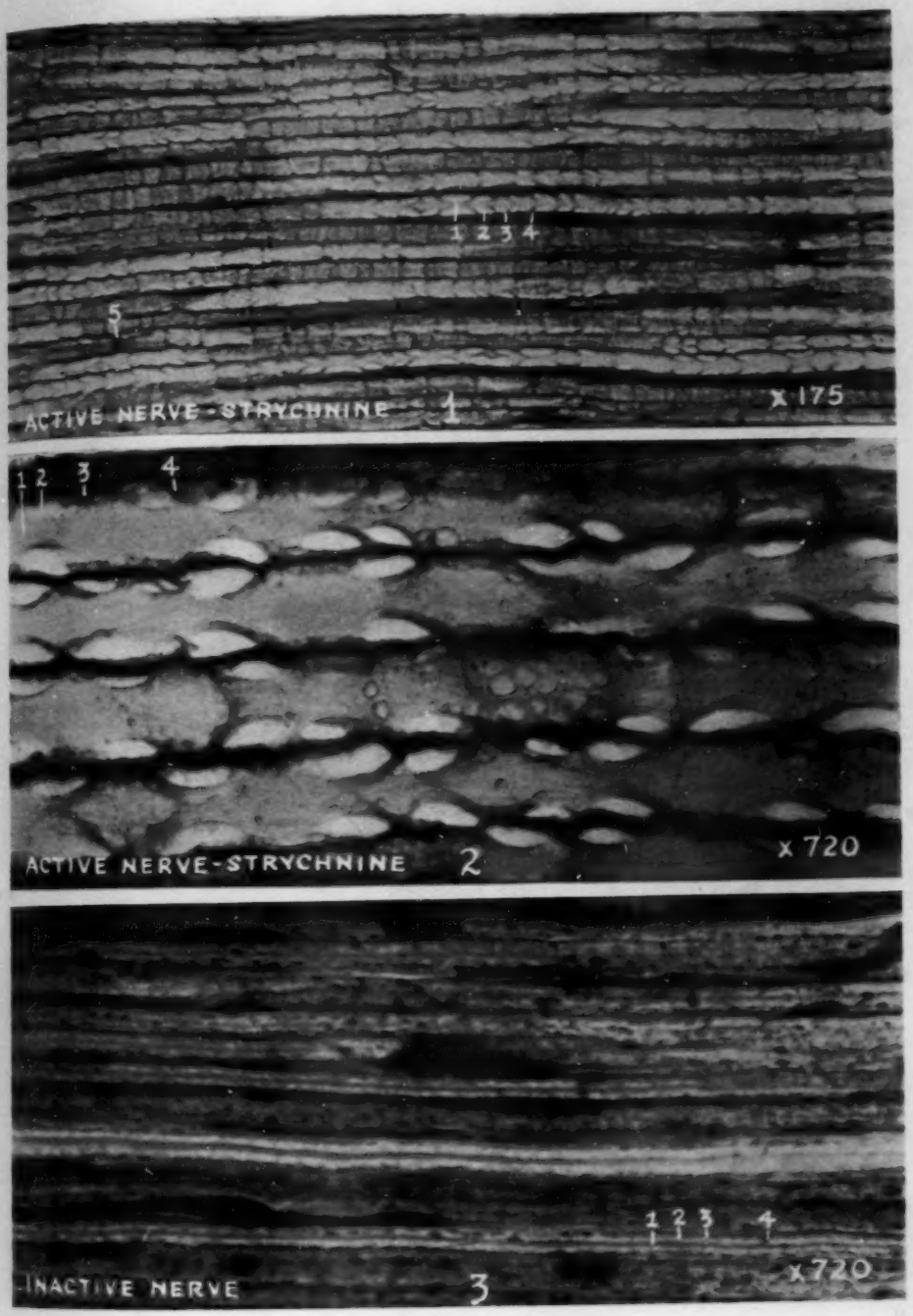
1 2 3 4
5
ACTIVE NERVE - STRYCHNINE 1
x 175
1 2 3 4
ACTIVE NERVE - STRYCHNINE 2
x 720
1 2 3 4
INACTIVE NERVE 3
x 720

PLATE I

PLATE II—EXPLANATION OF FIGURES 4 AND 5

Fig. 4.—Photomicrograph (untouched) of a longitudinal section of the tenth motor nerve root of a cat (Felis domestica) after it had been subject for four hours to the action of ethyl carbamate (given with stomach tube) and then for three hours to ether anesthesia, the cat being then given a lethal dose of ether; × 720; formaldehyde-thionine-dioxane technic. The small numbers *1, 2, 3, 4* and *5* have the same indications as in figure 1. The outline of the cylindric wall of each axon is relatively uniform, with a narrow layer of myelin between it and the outer neurilemma. The ratio of myelin to axon, 1 : 6, approaches that found by de Renyi in his microdissection studies. The outlines of the axons are relatively undisturbed. The relaxation of muscles under deep anesthesia and absence of pain or analgesia are associated with absence of and decrease in the number of nerve fibers with the beaded deformation of the axon. The number of axons that have pressure pulses is greatly reduced in the nerve fiber after prolonged deep anesthesia.

Fig. 5.—Photomicrograph (untouched) of a longitudinal section of the tenth motor nerve root of a cat (Felis domestica) killed during the initial stage of excitation with a lethal dose of ether; × 720; formaldehyde-thionine-dioxane technic. There is a nervous spectrum in the liquid axon of radiant pressure energy, composed of brief pressure pulses of the same or different wavelengths and histologically expressed as a series of alternate dilatations and constrictions. The dilatations are zones of compression, and the constrictions are zones of rarefaction, of longitudinal pressure waves transmitted through a confined liquid filament which is easily deformable. The characteristic serial expansions alternating with continuous constrictions of all of the axons of a nerve trunk are typical of nerve stimulation. The tonic and clonic contractions of all the muscular fibers, with multiplication in the number of the cross-striations, are associated with universal multiplication in the number of dilatations and constrictions of the axons of the nerve fibers under the influence of the initial stage of ether stimulation. There is generalized and synchronized wavelike deformation of all the axons underlying the augmentation of the excitability and conductibility of the various nerves involved in the initial stage of excitement of ether stimulation. The constrictions between the dilatation of the axons are not as great or as definite as those found after the administration of a lethal dose of strychnine.

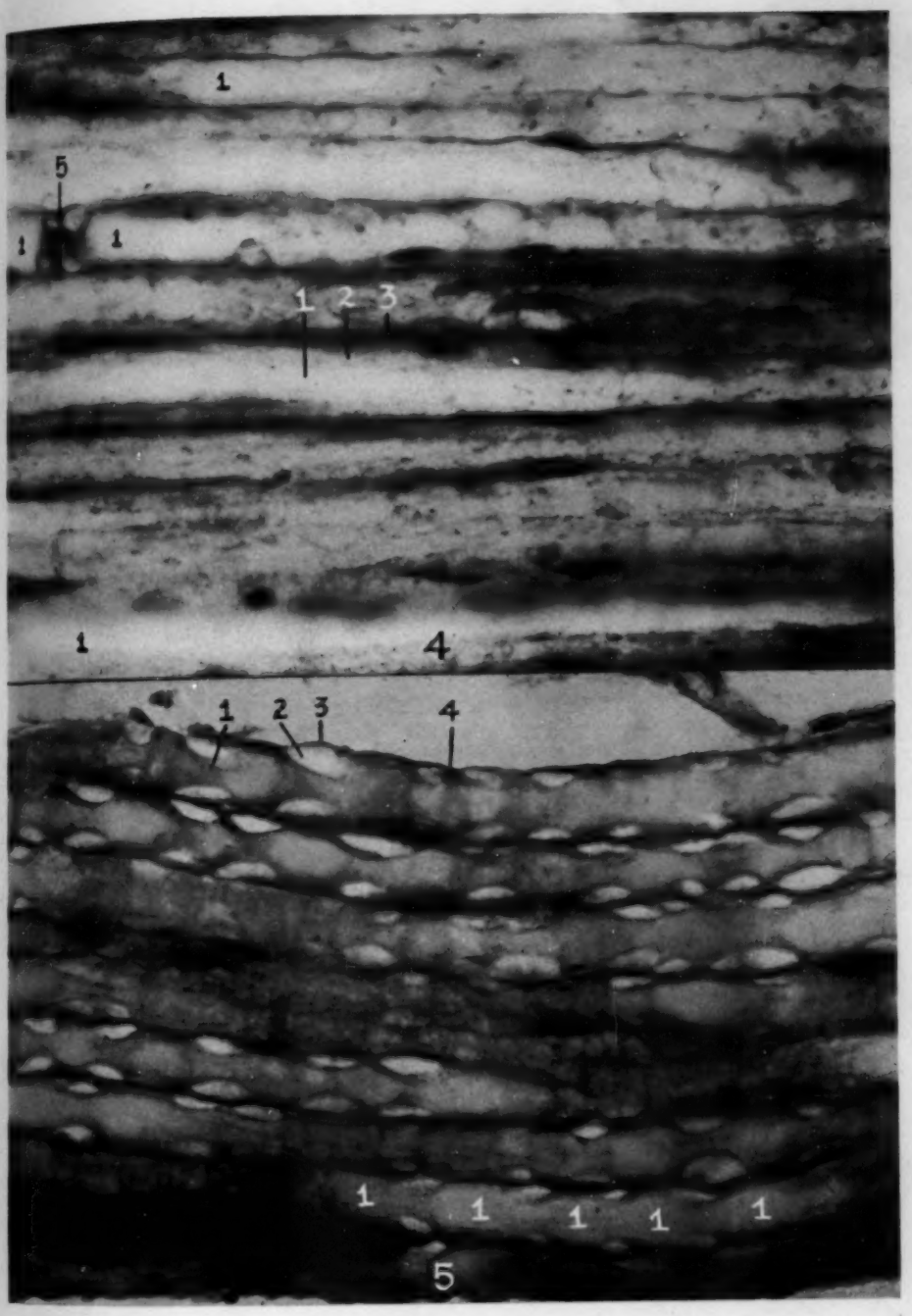

PLATE II

PLATE III—EXPLANATION OF FIGURES 6 AND 7

Photomicrographs (untouched) of longitudinal sections, 8 microns thick, of the eighth ventral motor root fibers of the cat (Felis domestica) killed with a lethal dose of strychnine; formaldehyde-thionine-dioxane technic used; fig. 6, × 175; fig. 7, × 720. The small numbers *1, 2, 3, 4* and *5* have the same indications as in figure 1. There is a nervous spectrum in the liquid axon (axis-cylinder) of radiant pressure energy, composed of brief pressure pulses of the same or different wavelengths and histologically expressed as a series of alternate dilatations and constrictions. The small pressure pulses of the axon, from 0.4 to 2 microns in length, are superimposed on the larger ones, from 10 to 50 microns in length. The dilatations are zones of compression, and the constrictions are zones of rarefaction, of longitudinal pressure waves transmitted through a liquid confined in an easily deformable microcapillary tubular wall. There is a generalized and synchronized pattern of the nerve pressure waves in the same and neighboring fibers. The myelin spaces are so evenly placed in relation to one another that a cross-striated effect is produced. There is a close similarity in the amplitude and wavelengths of nerve pressure expressed as dilatations of the axons. In some of the axons the localized increase of liquid pressure produced by strychnine stimulation is such that enormous bulgings occur. In certain places actual "blow outs" have taken place owing to the enormous intrinsic pressure within the liquid of the axon. In figure 6 these greatly enlarged lateral expansions appear black; in the sections they stain red.

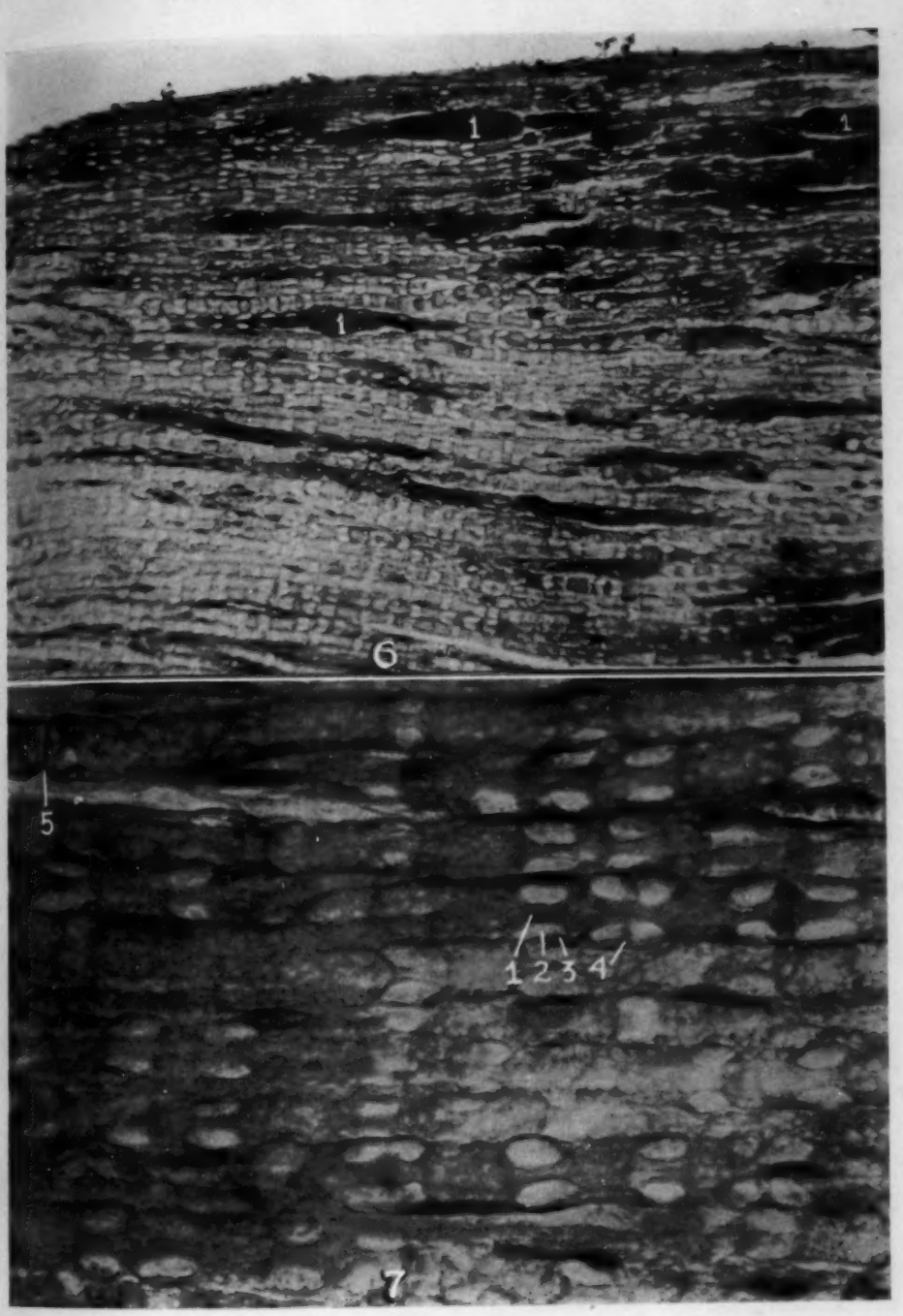

PLATE III

PLATE IV—EXPLANATION OF FIGURES 8 AND 9

Photomicrographs (untouched) of longitudinal sections, 8 microns thick, of the eighth ventral motor root fibers of a cat (Felis domestica) killed with a lethal dose of strychnine; formaldehyde-thionine-dioxane technic used; × 720. The small numbers *1, 2, 3, 4* and *5* have the same indications as in figure 1. There is a nervous spectrum in the liquid axon of radiant pressure energy, composed of brief pressure pulses of the same or different wavelengths and histologically expressed as a series of alternate dilatations and constrictions. The small pressure pulses of the axon, from 0.4 to 2 microns in length, are superimposed on the larger ones, from 10 to 60 microns in length. In some of the axons the localized increase of liquid pressure produced by strychnine stimulation is such that enormous bulgings occur. In certain places actual "blow outs" have taken place owing to the enormous intrinsic pressure within the liquid of the axon. In figure 8 these greatly enlarged lateral expansions appear black; in the sections they stain red. Two Schmidt-Lantermann adhesion bands (labeled *4*) are placed in opposite directions. They likewise may be directed either transversely, proximad or distad. The upper fiber of figure 9 has a constricted dark axon to the right which blends with the segmented dilatations of the axon toward the left.

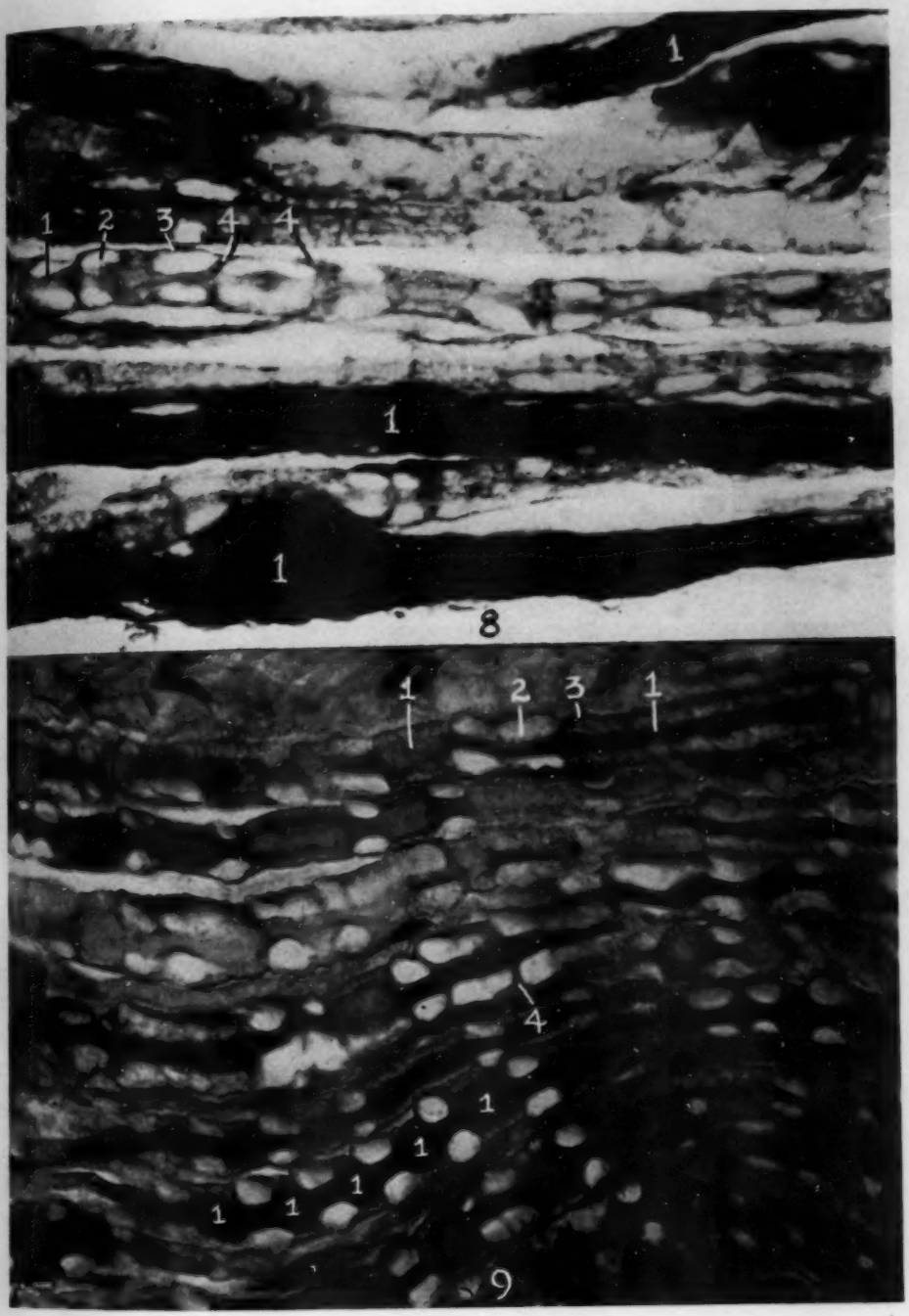

PLATE IV

PLATE V.—EXPLANATION OF FIGURES 10 AND 11

Photomicrographs (untouched) of longtitudinal sections, 8 microns thick, of the tenth dorsal sensory nerve root fibers of a cat (Felix domestica) killed with a lethal dose of strychnine; formaldehyde-thionine-dioxane technic; fig. 10, × 175; fig. 11, × 720. The small numbers *1, 2, 3, 4* and *5* have the same indications as in figure 1. There is a nervous spectrum in the liquid axon (axis-cylinder) of radiant pressure energy, composed of brief pressure pulses of the same or different wavelengths and histologically expressed as a series of alternate dilatations and constrictions. The small pressure pulses, from 0.4 to 2 microns in length, are superimposed on the larger ones, from 10 to 75 microns in length, structurally expressed as serial deformations of the axon. The axons have variable spectra of the nerve pressure waves. The active axons are in different phases, from direct expansive contacts with the neurilemma to small retracted dilatations or narrow tubes connected with the adhesion bands or Schmidt-Lantermann incisures. These bands extend from the axon to the neurilemma. The dilatations are of different lengths in the same and in neighboring fibers. The spaces occupied by myelin are of unequal sizes and shapes. The myelin is dissolved out of the nerve by dioxane. The myelin zones encircle the narrow contracted places of the axons between dilatations or between Schmidt-Lantermann bands of adhesions.

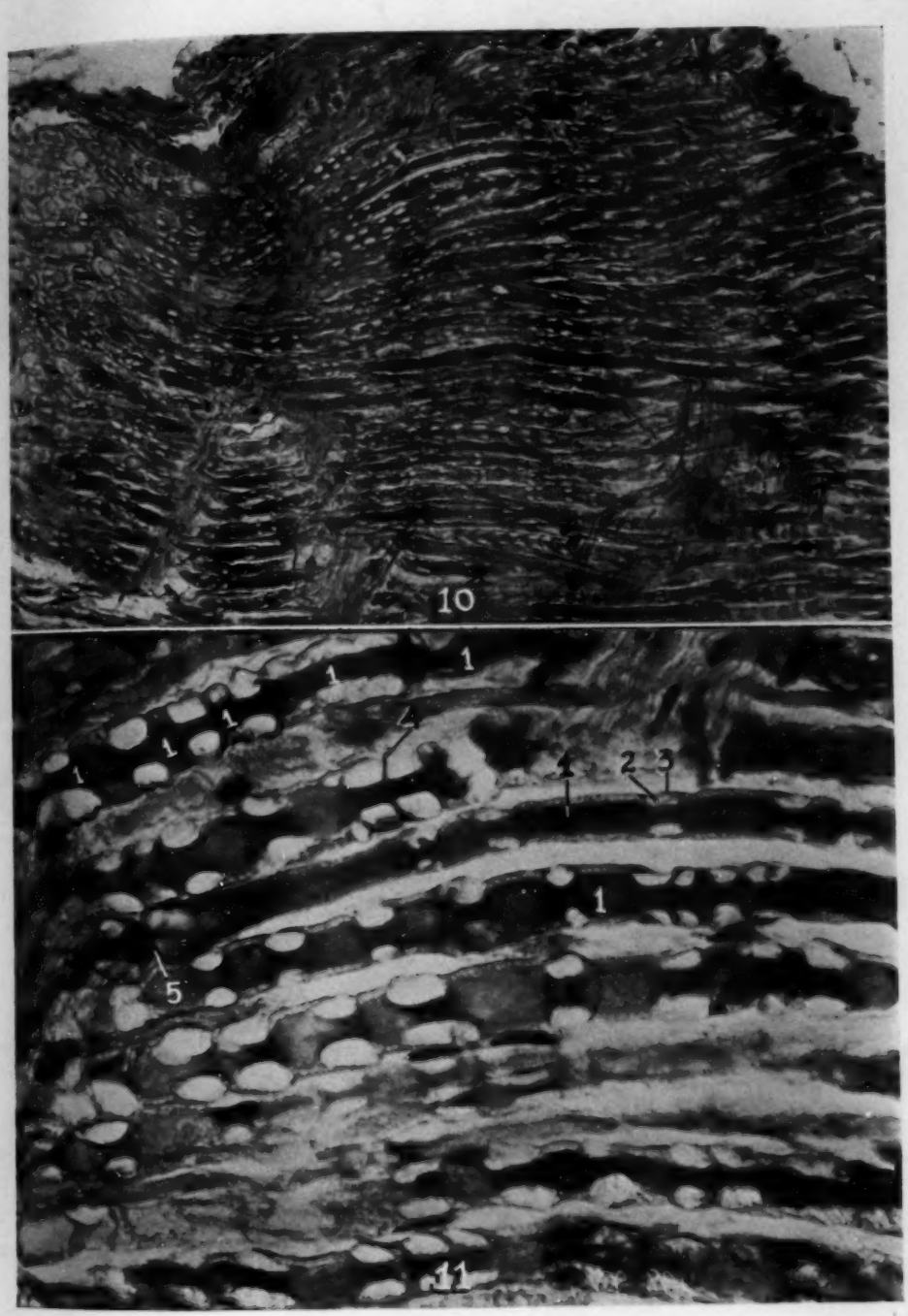

PLATE V

PLATE VI—EXPLANATION OF FIGURES 12 AND 13

Photomicrographs (untouched) of longitudinal sections, 8 microns thick, of the eighth ventral motor nerve root fibers of a cat (Felis domestica) killed by a lethal dose of strychnine; × 720; formaldehyde-thionine-dioxane technic used. The small numbers *1, 2, 3, 4* and *5* have the same indications as in figure 1. There is a nervous spectrum in the liquid axon (axis-cylinder) of radiant pressure energy, composed of brief pressure pulses of the same or different wavelength and histologically expressed as a series of alternate dilatations and constrictions of the microcapillary inner tube of the nerve fiber. The small pressure pulses of the axon, from 0.4 to 2 microns in length, are superimposed on the larger ones, from 10 to 75 microns in length. In the lower part of figure 12 there are cross-sections of the nerve fibers. The sizes of the axons differ, depending on whether or not the cross-section passes through a dilatation or a constriction. The lower part of figure 13 has an axon in longitudinal section, which is constricted at the left and merges toward the right into the alternate dilatations and constrictions of variable lengths. This is a spectrum or image of pressure waves expressed in the liquid of the axon with the characteristic accompanying deformations emphasized by strychnine stimulation. The difference between a normal impulse and the augmented one produced by strychnine is a quantitative one of intensity, frequency and number of fibers involved. The augmented kinetic energy of the train of nerve pressure waves is transferred to and absorbed by the muscles. The tetanic sustained contractions of the muscles are due to the increased intensity of the radiated nerve pressure.

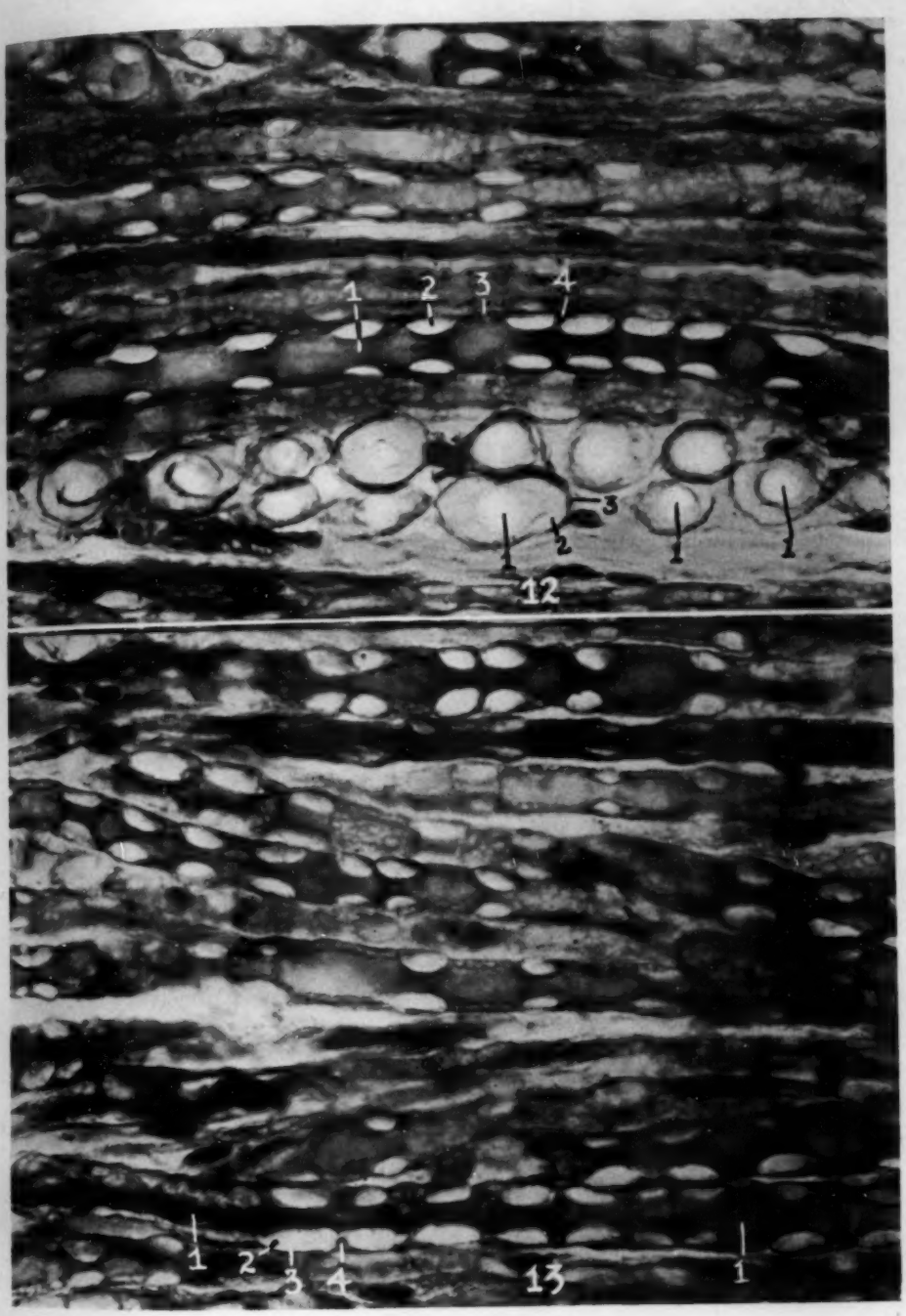

PLATE VI

into the regions overlying and encircling the constricted places of the axis-cylinder. The large dilatations are from 5 to 75 microns in length. There are ripples from 0.5 to 2 microns in length superimposed on the peripheries of the large dilatations. The dilatations are funnel shaped, spherical, cylindric with tapering ends and arrowhead shaped.

When the axis-cylinder is narrow and retracted from the neurilemma, periodic bands occur along the former line of adherence between the dilatations of the axis-cylinder and the neurilemma. These bands form segments of the myelin and are the inconstant Schmidt-Lantermann incisures. These may be directed laterad, proximad, distad and in opposite directions (figs. 8, 9, 11, 12 and 13). The directions are influenced by the phase differences of microcapillary waves of pressure. The myelin is a passive lipoidal liquid. Its structural pattern is determined by the dynamic external framework of the inner tube of the nerve, the active axis-cylinder. When the summits of the serial dilatations of the hyperactive axonic inner tube come in direct contact with the neurilemmal outer tube, there is a serial segmentation of the myelin. The myelin is dissolved out of the nerve by dioxane. The myelin spaces are round, oval and flat spaces encircling the constrictions between the periodic swellings of the axis-cylinder (figs. 10 to 13). The myelin is likewise segmented by the temporary, evanescent bands of adhesion, the Schmidt-Lantermann incisures.

SUMMARY

The different degrees of nervous activity have corresponding structural patterns of the axis-cylinder of the myelinated nerve fiber with a neurilemma. Abnormal increased action of a nerve is characterized by multiple wavelike structures in most of the component axis-cylinders. These structural deformations are abolished by complete inactivity. There are gradations between these two extremes of action and inaction with a corresponding correlation between structure and function of nerves. Strychnine establishes synchronism of the histologic patterns of dilatations and constrictions in most of the axis-cylinders within a nerve fiber. These wavelike structures are greater in number and size in the axis-cylinder of a nerve fiber from an animal killed by strychnine than in the normal nerve. Furthermore, a greater number of the axis-cylinders in a strychnized nerve have wavy deformations than of those in the normal nerve. Prolonged deep anesthesia diminishes the structural expression of nervous function; the axis-cylinders become smoother and more uniform in contour than normal by the elimination of the wavelike structures.

These histologic wavelike structures of alternate dilatations and constrictions of the actively conducting axis-cylinders are tentatively considered as the representation of a train of microcapillary longitudinal waves of colloidal pressure transmitted through a fine filament of col-

loidal liquid neuroplasm. Various stimulating and depressing agents may increase or decrease the generation and projection of these pressure waves. According to this hypothesis of the wave mechanics of nerve action and the photomicrographic demonstrations herein reported there is no constant internal structure of nerve fibers. A fixed structure of myelinated nerves is taught at the present time in textbooks of histology.

The multiple changes of the internal architecture of a myelinated nerve fiber with a neurilemma have a tentative explanation in the light of the variable frequencies, amplitudes and phases of action of the transmitted waves of pressure. Whether this dynamic explanation is true or false, there exists irrefutable evidence that there are concomitant characteristic histologic symbols of differential protoplasmic nerve action which have been overlooked by anatomic investigators.

The nerve impulse is transmitted through the entire axis-cylinder, which is in a liquid state. Neurofibrils are not the specific structural conductors of nerve action. The neurofibrils are considered as longitudinally condensed parts of the colloidal liquid of the axon drawn out into a microcapillary filament by the tension of relative growth. The neurofibrils are inconstant structures that vary with the physical state and shape of the axis-cylinder: they are the physical resultants of longitudinal tension and lateral compression in a protoplasmic strand drawn out in extension comparable to the physical conditions that form myofibrils in muscle.

The conclusion is tentatively made that the variable explosive chemical changes within living protoplasm produce either regular or irregular mechanical explosion waves of intrinsic colloidal pressure. The differential anatomic arrangements and metabolic rates of the living protoplasm determine the variable patterns assumed by these mechanical waves of pressure in the ameba, the muscle and the nerve fiber. These mechanical pressure waves of explosive chemical action within protoplasm produce the histologic signs of ameboid, muscular and nervous actions. They are expressed histologically as relatively regular cross-striations and intercalated disks in branched heart muscle fibers and as dilatations and constrictions in the liquid microcapillary axon of the active myelinated nerve fiber with a neurilemma. In the ameba, unattached and inconstant in form, the colloidal explosion waves of pressure are irregular and inconstant. These give a variable, alveolar, filar, hyaline or granular structure to protoplasm. The arrangement of the spongioplasm and hyaloplasm in the ameba is assumed to be determined by the intrinsic wave mechanics of colloidal pressure caused by the explosive chemical changes in the living protoplasm. Mechanical explosion waves of living colloidal protoplasm underlie the mechanical interdependence by resonance of the functional muscular and nervous systems. In this study is demonstrated a correlation between nerve structure and function which heretofore has not been detected.

AURICULAR THROMBOSIS IN RHEUMATIC HEART DISEASE

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Although there have been extensive studies[1] of the acute and chronic inflammatory lesions encountered in the auricles of the heart in rheumatic fever, there have been no detailed reports concerning the thrombi occurring in them. In an earlier investigation of the vascular lesions underlying bland thrombi of the aorta and coronary arteries, Clark, Graef and Chasis[2] frequently encountered fibrinous deposits in the substance of the intimal plaques. These had such an intimate relation to the thrombi as to suggest that they might furnish the soil for progressive formation of thrombi. Since some of the fibrin-like masses noted frequently in the walls of vessels, endocardium, cardiac valves and supporting tissue elsewhere in rheumatic fever, and variously attributed to local changes in collagen or to infiltration by elements of the blood, appear similar to lesions encountered in the vessels of non-rheumatic specimens, a study of the thrombi in rheumatic hearts was undertaken to determine the nature of the underlying endocardial changes.

Other observers have referred to such lesions as fibrinous (Mallory[3]) or fibrinoid (Neumann,[4] Jäger,[5] Leary,[6] Karsner and Bayless,[7]

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(Footnotes continued on next page)

Jucker[8]). Some have interpreted such lesions to represent altered or necrotic collagen (Jäger,[5] Leary,[6] Jucker[8]). In the study[2] earlier reported, no evidence was found to support this view, and such so-called fibrinoid deposits were attributed to fibrin and other elements of the blood. Attention has been paid in the present study, therefore, to the differentiation of swollen collagen from fibrinous deposits.

Since earlier clinicopathologic correlations were made only on indirect evidence, the effects of alteration in the hemodynamics (resulting from valvular deformity, cardiac arrhythmia and congestive heart failure) have been weighed as well as the morphologic findings in our consideration of the pathogenesis of the thrombi found.

MATERIALS AND METHODS OF STUDY

Pathologic Criteria.—The criteria followed here have been essentially the same as those outlined in a previous communication from this laboratory.[9] The term "rheumatic heart" designated any specimen in which (exclusive of specific bacterial inflammatory or atherosclerotic change) there was evidence of valvulitis of a varying degree in association with serosal, interstitial or vascular inflammation or their end-results.

Rheumatic activity was indicated by typical Aschoff bodies or active (non-bacterial) valvulitis, either superficial or deep. Interstitial perivascular cellular foci, ranging from Aschoff nodules to aggregates of lymphocytes, histiocytes, plasma cells and polymorphonuclear leukocytes, and fresh vascular lesions in association with carditis also were regarded as signs of activity. Scarred deformed heavily vascularized valves, intrafascicular and perivascular myocardial fibrosis, and healed pericarditis and aortitis (when associated with typical lesions elsewhere) were considered as evidence of an inactive, healed rheumatic inflammation.

The degree of mitral stenosis was estimated by a consideration of the presence or absence of free motion of the valve, the flexibility of the leaflets, the extent of fusion of the commissures and the comparative size of the valve and ring orifices.

During the past fifteen years, 178 hearts obtained at autopsy from the Third (New York University) Division of Bellevue Hospital conformed to these criteria. Twenty-four of the 178 specimens exhibited thrombi, and it was this group which formed the basis of the present study.

Fixation in solution of formaldehyde U. S. P. was used throughout. In addition to the blocks suggested by Gross, Antopol and Sacks,[10] two or three representative portions of the thrombus were taken. The paraffin-embedded material was sectioned at 6 microns. The hematoxylin-eosin stain, Weigert's stain for elastic tissue combined with Van Gieson's stain, Mallory's phospho-

5. Jäger, E.: Virchows Arch. f. path. Anat. **284**:526 and 584, 1932.

6. Leary, T.: Arch. Path. **17**:453, 1934.

7. Karsner, H. T., and Bayless, F.: Am. Heart J. **9**:557, 1934.

8. Jucker, P.: Virchows Arch. f. path. Anat. **295**:301, 1935.

9. de la Chapelle, C. E.; Graef, I., and Rottino, A.: Am. Heart J. **10**:62, 1934.

10. Gross, L.; Antopol, W., and Sacks, B.: Arch. Path. **10**:840, 1930.

tungstic acid-hematoxylin stain and Lepehne's benzidine method [10a] were employed as a routine in all cases. In almost every instance, silver stains (method of Foot and Foot) counterstained with trinitrophenol-fuchsin (Van Gieson's solution) were made. Specimens were examined for bacteria by the Gram-Weigert technic. In a few selected cases, the thrombi were sectioned serially.

ANALYSIS OF MATERIAL

Incidence.—Twenty-four of the 178 hearts showing rheumatic disease had thrombi in the auricles or in their appendages—an incidence of 13 per cent. No intraventricular thrombi were encountered.

Age.—The average age of the patients from whom these twenty-four hearts came was 47 years, with a range from 7 to 74, and with 75 per cent of the patients in the fourth to seventh decades of life, inclusive; 49 years was the average age for the fourteen who had auricular fibrillation; 43 years, for the eight with sinus rhythm.

Sex.—There were fourteen male and ten female patients.

Rhythm.—Fourteen patients had persistent auricular fibrillation [11]; of these, ten were male and four were female. Eight had sinus rhythm; of these, three were male and five, female. There was one who had varying rhythm (sinus rhythm, auricular fibrillation and flutter) and there was another who had complete heart block.

Congestive Heart Failure.—Evidence of moderate or severe failure (in the form of edema, effusions into the serous sacs and passive congestion of the viscera) was present at necropsy in twenty-one patients. The remaining three patients of the series had histories of congestive failure.

Location of the Thrombi and Their Relationship to the Gross Endocardial Change and to Mitral Stenosis.—In fourteen of the twenty-four hearts the thrombi were confined to the left side, in five to the right, and in the remaining five they were bilateral. In no instance was a thrombus found in the chamber of the right auricle proper. Gross auricular scarring of a severe degree was present in all but two specimens. In every instance, the left auricle was affected more severely than the right. A "MacCallum patch" [12] was noted in every heart but two and in five these patches were the sites of thrombosis. In the two specimens excepted there was minimal scarring in the auricles, and in one it was associated with mitral stenosis of a very mild degree. The thrombi in both were recent structures. In one heart with bilateral

10a. Lepehne, G.: Beitr. z. path. Anat. u. z. allg. Path. **65**:163, 1919.

11. Auricular fibrillation, verified by electrocardiograms, was present in every case for at least three weeks prior to death.

12. The term "MacCallum patch" is used in its broadest sense to include gross scarring above the posterior mitral leaflet.

thrombosis, the endocardium of the right auricular appendage was fairly thin, and the thrombus was recent; the left auricle showed fairly severe sclerosis. In four hearts deposits of calcium were noted in the endocardium. Gross hemorrhage was not observed.

In only two of the five hearts with thrombi limited to the right side was there severe deformity of the mitral valve, and one of these also showed moderate stenosis of the tricuspid valve. In all five specimens with thrombi on both the right and the left side there was severe mitral stenosis. Of the fourteen specimens in which the thrombus occurred in the left auricle alone, eleven showed severe mitral stenosis. Thus, in sixteen of the nineteen hearts in which a thrombus occurred in the left auricle there was severe mitral stenosis. In two with slight mitral deformity there were very severe aortic stenosis and insufficiency (table).

Disorders of Rhythm.—Auricular fibrillation had been present in fourteen of the patients, and in eleven of these it was associated with severe mitral stenosis. If the one with paroxysmal fibrillation and the one with complete auriculoventricular block are included, sixteen of the twenty-four patients (two-thirds) had cardiac rhythmic disorders before death. In ten of the fourteen patients with thrombi limited to the left side there was auricular fibrillation, whereas it occurred in only one of the five with thrombi limited to the right side.

Endocardial Changes in Relation to the Thrombus.—The gross appearance of the thrombi was fairly uniform; in most cases there was presented a mottled grayish red mass, rather firmly attached at the base but friable in the peripheral region. The fourteen thrombi confined to the auricular appendages were ovoid and measured about 1 by 2 by 2 cm. In the cases in which the left auricle proper was involved, the thrombi varied from an area of 5 sq. mm. to a pedunculated mass 8 cm. in diameter.

Every specimen was examined for evidence of recent and old rheumatic inflammation. The auricular endocardium beneath the thrombus and in the control blocks was searched for foci of superficial and deep endocarditis, scarring, hemorrhage and calcification. The presence of deeply eosinophilic fibrinoid material like that described by Klinge [13] as a swelling of the "ground substance" and considered by him to be an essential lesion in active rheumatic inflammation was sought in the endocardium and in relation to the thrombus.

1. Cases with Active Carditis: In 58 per cent of the cases (fourteen of twenty-four) evidences of carditis, either diffuse or focal, were found. Active inflammation in the auricular endocardium beneath

13. Klinge, F.: Ergebn. d. allg. Path. u. path. Anat. **27**:1, 1933.

Analysis of Findings in Twenty-Four Rheumatic Hearts with Auricular Thrombi

Case	Rhythm	Location of Thrombus	Degree of Mitral Stenosis	Auricular Endocardium Beneath Thrombi*				
				Active Inflammation	Sclerosis	Hemorrhage	Calcification	Fibrinoid
		Thrombi on Left Side Only						
1	Auricular fibrillation	Left auricle	Severe	Y	Y	N	N	Y
2	Auricular fibrillation	Left auricle	Severe	N	Y	N	Y	Y
3	Auricular fibrillation	Left auricle	Severe	N	Y	N	Y	Y
4	Auricular fibrillation	Left auricular appendage	Severe	Y	Y	N	N	Y
5	Auricular fibrillation	Left auricular appendage	Moderate†	N	Y	N	N	Y
6	Auricular fibrillation	Left auricular appendage	Severe	Y	Y	N	N	Y
7	Auricular fibrillation	Left auricular appendage	Severe	N	Y	N	N	N
8	Auricular fibrillation	Left auricle and left auricular appendage	Severe‡	Y	Y	N	N	Y
9	Auricular fibrillation	Left auricle and left auricular appendage	Mild†	Y	Y	N	N	N
10	Auricular fibrillation	Left auricle and left auricular appendage	Severe	N	Y	N	Y	Y
11	Regular sinus rhythm	Left auricle	Severe	Y	Y	N	N	Y
12	Regular sinus rhythm	Left auricular appendage	Severe	Y	Y	N	N	N
13	Regular sinus rhythm	Left auricular appendage	Mild	N	Y	N	N	N
14	Complete block	Left auricle and left auricular appendage	Severe	Y	Y	N	N	Y
		Thrombi on Right Side Only						
15	Auricular fibrillation	Right auricular appendage	Mild	N	N	N	N	N
16	Regular sinus rhythm	Right auricular appendage	Moderate	N	Y	N	N	N
17	Regular sinus rhythm	Right auricular appendage	Mild	N	Y	N	N	N
18	Regular sinus rhythm	Right auricular appendage	Severe	N	N	N	N	N
19	Varying§	Right auricular appendage	Severe#	N	Y	N	N	N
		Bilateral Thrombi						
20	Auricular fibrillation	Right and left auricular appendage	Severe	N	Y	N	N	N
21	Auricular fibrillation	Right and left auricular appendage	Severe#	N	Y	Y	N	N
22	Auricular fibrillation	Right auricular appendage; left auricle and left auricular appendage	Severe	N	Y	N	N-Y	Y
23	Regular sinus rhythm	Right and left auricular appendage	Severe	N-Y	Y	N-Y	N	N-Y
24	Regular sinus rhythm	Right auricular appendage; left auricle and left auricular appendage	Severe	N-Y	N-Y	N	N	N

* Y means yes; N, no. N-Y refers to the findings in the respective thrombi.
† There was also severe aortic stenosis.
‡ There was also adhesive pericarditis.
§ Sinus rhythm, auricular flutter and fibrillation were noted.
# There was also severe tricuspid stenosis.

the thrombus was demonstrated in ten (42 per cent). In ten cases the thrombi involved the auricular appendages, and activity was found in the endocardium beneath the thrombi in seven of these, and these thrombi were all in the left auricular appendages.

The type of active inflammation found beneath the thrombi varied from case to case. In only one instance (fig. 4) was typical superficial inflammation encountered; it was superimposed on a thickened, vascularized endocardium and subendocardium. In this instance, in which

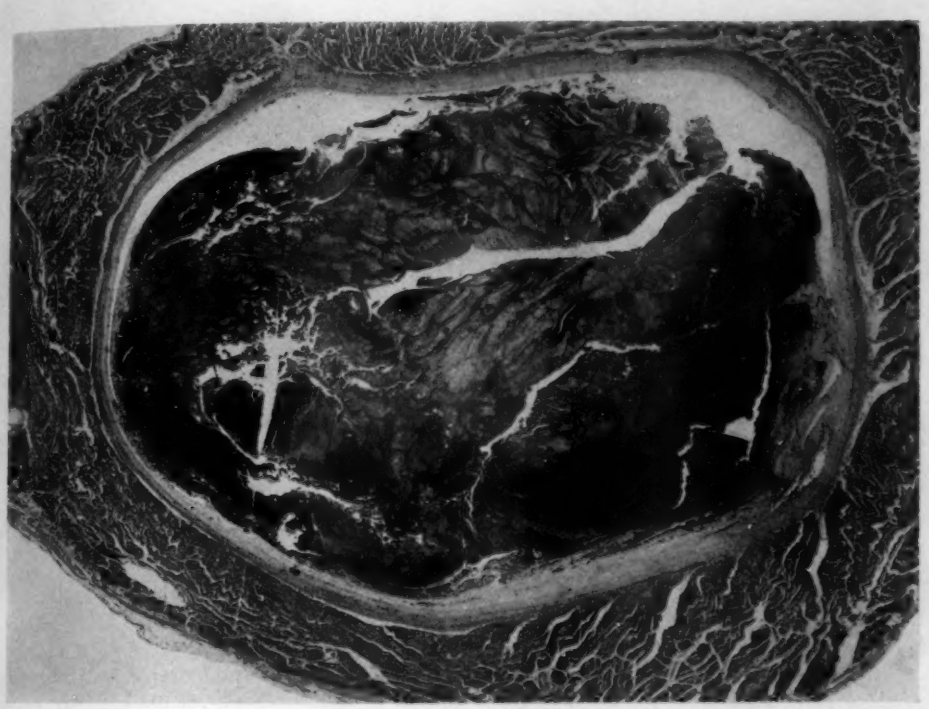

Fig. 1.—A 29 year old white man with a history of auricular fibrillation died in congestive heart failure. The heart showed severe mitral stenosis and pancarditis. The cross-section of the left auricular appendage illustrates the built-up nature of the thrombus; the older lesions have finger-like extensions into the endocardium. The wavy overlapping platelet columns are shown in the center of the thrombus. Some of the clefts are unavoidable artefacts. Hematoxylin and eosin stain.

the thrombus occurred above the posterior mitral leaflet, the entire endocardium was markedly thickened by collagenous and elastic tissue containing numerous new capillaries, plasma cells, lymphocytes and large basophilic mononuclear cells. Some of the latter were caught between lamellae of the elastica and compressed into rows, one above the other.

At one site a dense homogeneous mass of material which had a strong affinity both for eosin and for the trinitrophenol stains was deposited on the intimal surface. This material also stained and appeared like fibrin with Mallory's phosphotungstic acid-hematoxylin stain. A few thin shreds of material with similar tinctorial properties were also noted in the endocardium beneath this deposit, and at several sites continuity with the superficial mass was demonstrated.

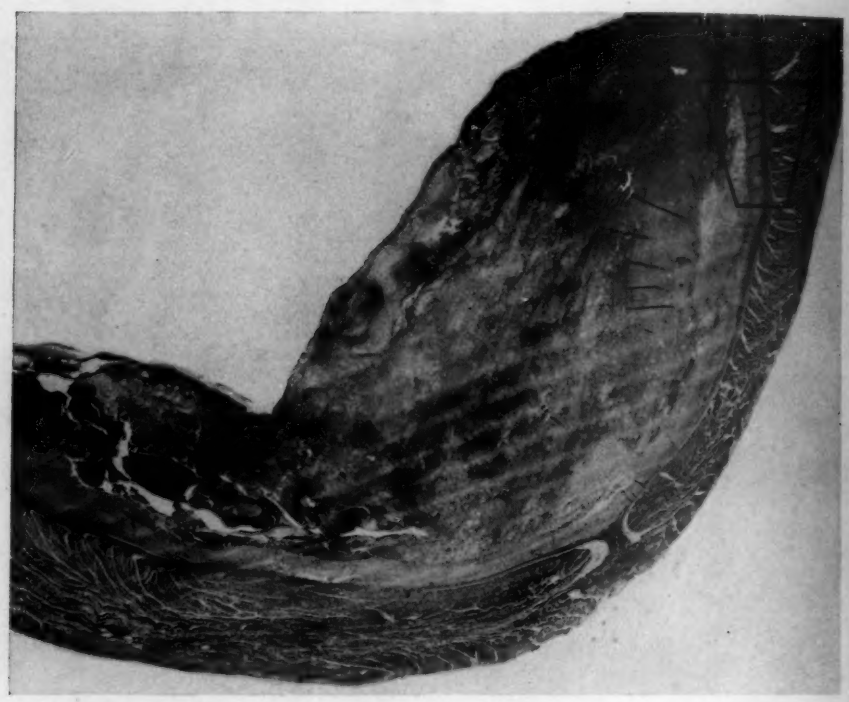

Fig. 2.—A 63 year old white man who suffered from auricular fibrillation died in congestive heart failure. The heart showed severe mitral stenosis and pancarditis. A low power photomicrograph is shown of a section of the thrombus found attached to the posterior wall of the left auricle. The compressed lamellated appearance and association with endocardial fibrosis are shown plainly. Extensions of the compressed fibrinous masses were found all along the base. See figure 10 for a higher magnification of the field outlined in black ink. Hematoxylin and eosin stain.

The evidences of activity were residual collections of lymphocytes, histiocytes and, rarely, large basophilic mononuclear cells. Extensive vascularization of the scarred underlying endocardium was conspicuous in all lesions, and the cellular aggregates were usually encountered close to the myocardial junction, most often in the deepest layers of the

thickened endocardium. In some cases, the lesions were hard to find. In one, serial section of a single block revealed evidence of activity. In another instance (figs. 5, 6 and 7) in a block bordering on the endocardial thrombus two zones of eosinophilic swelling of collagen were noted together with large mononuclear cells compressed between the lamellae of the elastica. Two other zones of collagenous swelling without cellular infiltration were found in the same section. In another

Fig. 3.—A 31 year old white man with regular sinus rhythm and severe mitral stenosis died in congestive heart failure. The heart showed active rheumatic endocarditis and myocarditis as well as evidence of much previous damage. The cross-section of the left auricular appendage shows a large thrombotic lesion with evidences of the progressive nature of the process. The base exhibits extensive organization with conspicuous new vessel formation. Toward the surface fresher thrombus formation is discernible. The darker regions represent masses of red blood cells; the lighter regions, compressed masses of fibrin. Hematoxylin and eosin stain.

case, sections of the thrombosed auricular appendage (fig. 8) revealed superficial inflammatory changes of the endocardium beneath the thrombus, consisting of lifting of the endocardium, multiplication of endothelial cells and infiltration by a few polymorphonuclear leukocytes.

On tracing the endocardium around the appendage (fig. 9) a fresh area of subendocardial inflammation was found, showing eosinophilic swelling of collagen and rows of large mononuclear cells compressed between lamellae of the elastica. The three instances of auricular endocarditis in association with formation of thrombi were the only ones in which definite swelling of collagen was shown. On staining these sections differentially with Mallory's phosphotungstic acid-hematoxylin stain and Van Gieson's stain, it was found that the swollen collagenous bundles retained their affinity for fuchsin in the latter stain and stained like

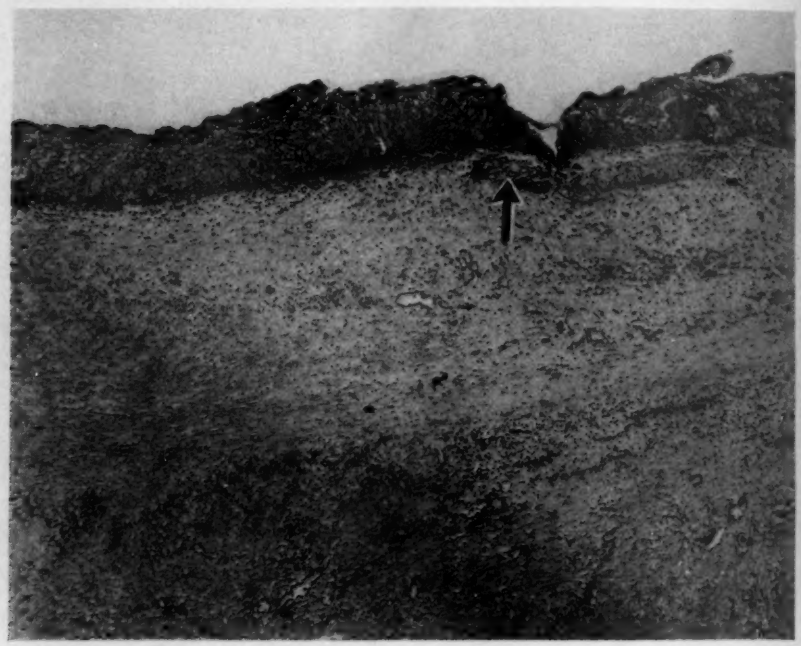

Fig. 4.—A 10 year old Negro had repeated episodes of rheumatic carditis. Death occurred during recurrence and in congestive heart failure. The heart showed severe mitral stenosis and pancarditis. The photomicrograph shows a section through a visible thrombus found on the posterior wall of the left auricle. Note fresh deposits of blood elements on and in the inflamed endocardium. Evidence of previous damage is seen in the diffuse thickening of the subendocardial tissue, with new vessel formation. The arrow indicates a zone of fibrin-staining material located in the thickened endocardium. It is deeply eosinophilic but stains like fibrin with Mallory's phosphotungstic acid-hematoxylin stain.

collagen with the phosphotungstic acid-hematoxylin stain. Of the remaining four cases of auricular thrombosis, the condition was associated with active valvular endocarditis in two, with active focal pericarditis in one and with active myocarditis and pericarditis in one.

There were two examples of bilateral thrombosis in which activity was found only beneath the thrombi in the left auricular appendages.

The endocardium exhibited a varying, usually severe, degree of fibrous scarring. The only exception to this finding was the normal endocardium beneath the fresh thrombus in the right auricular appendage in one case.

Endocardial hemorrhage was present beneath the thrombus in two cases. In one of these no fresh activity could be demonstrated beneath the thrombus, although there was accompanying active valvular endocarditis. In the second case there were signs of activity in the vicinity of the hemorrhage.

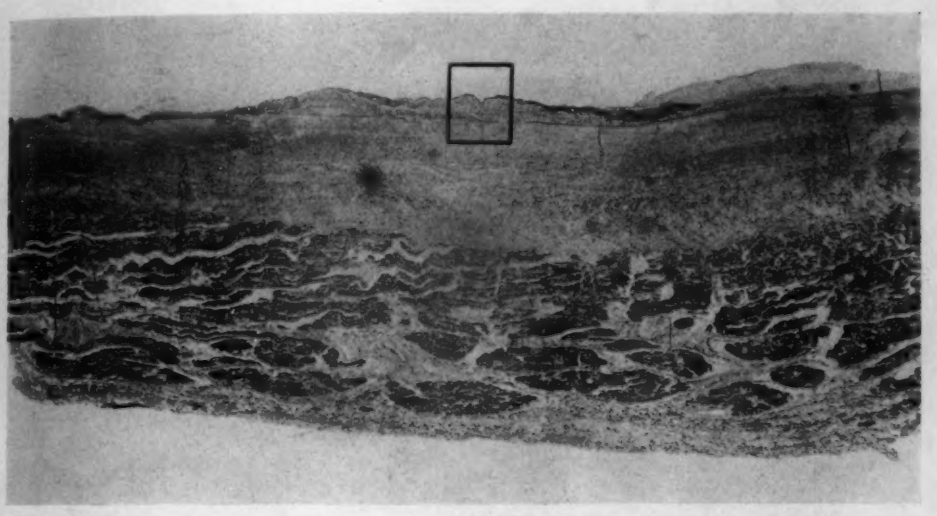

Fig. 5.—A low power photomicrograph of a section of the left auricle from the same case as the section shown in figure 2, showing extensive endocardial sclerosis and myocardial fibrosis of the rheumatic type. On the surface are seen fibrinoid deposits with extension into the deeper layers, which are deeply eosinophilic but which stain like fibrin by Mallory's phosphotungstic acid-hematoxylin method. In the same section regions of focal collagenous swelling are observed. (See fig. 6.)

Calcification occurred once in an inactive scarred endocardium.

Fibrinoid material was present in the endocardium (figs. 1, 2, 4, 5 and 10) and was found extending into the thrombi in nine of the fourteen cases with carditis. In two instances in which it was present, there were no signs of endocardial inflammation. In all the other cases in which fibrinoid material was seen there was evidence of active inflammation in the endocardium. Of the seven cases in which fibrinoid material was absent from the thrombi, three showed endocardial activity

and four did not. In one case, although fibrinoid material was not seen in direct relationship to the thrombi, it was demonstrated elsewhere in the left auricular endocardium of the same specimen.

With three exceptions, all the thrombi in this group were progressively laminated bland structures. In the superficial layers they were composed of varying proportions of platelets, polymorphonuclear

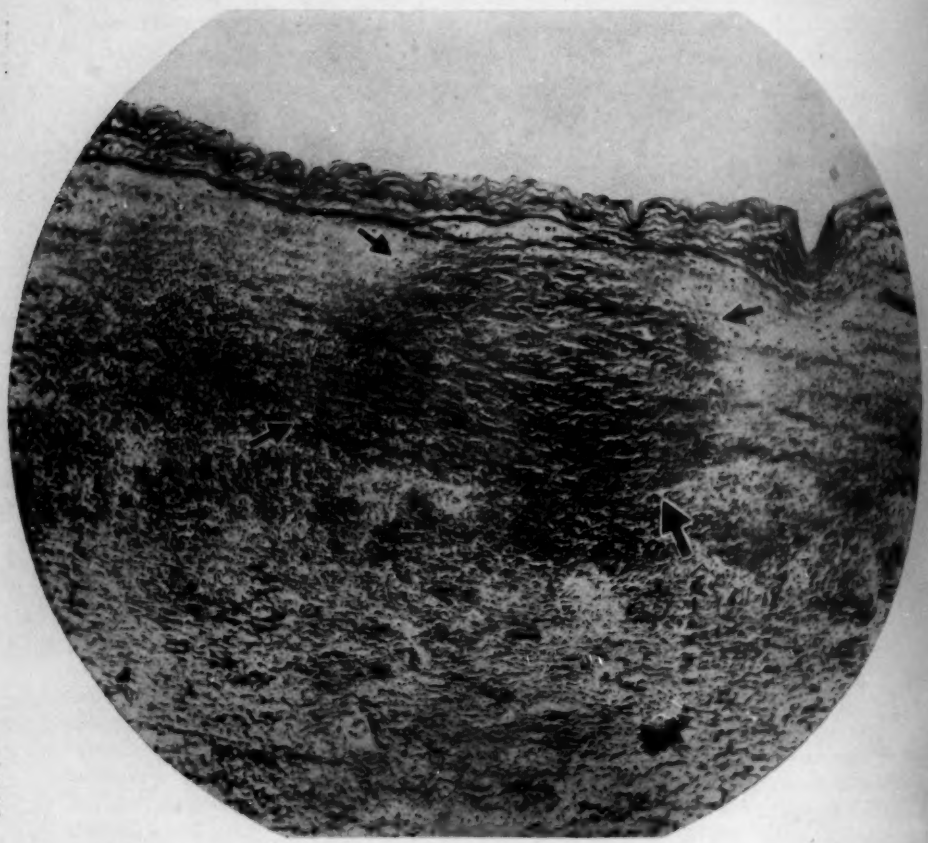

Fig. 6.—A photomicrograph of a portion of the field indicated by the box in figure 5 (stain, phosphotungstic acid-hematoxylin). The roughly spindle-shaped dark zone (indicated by arrows) represents an area of swelling of collagenous fibers, which stained more deeply than normal collagen but not like fibrin.

leukocytes, red blood cells (intact and fused) and fine shreds of fibrin, and in the deeper zones there were condensed and compressed layers or masses of hyalinized fibrin. Blood pigment, both extracellular and within macrophages, was seen occasionally near the base. The organization at the endocardial junction varied from minimal to almost com-

plete replacement of the original thrombotic elements by dense scar tissue. In such instances the original endocardium could be identified only if its elastica was distinguished. As a rule, when fibrinoid material was present in the endocardial base of the thrombus, it occurred in the form of condensed streamers, from which fine strands could be traced into the overlying thrombus. However, continuity of the endo-

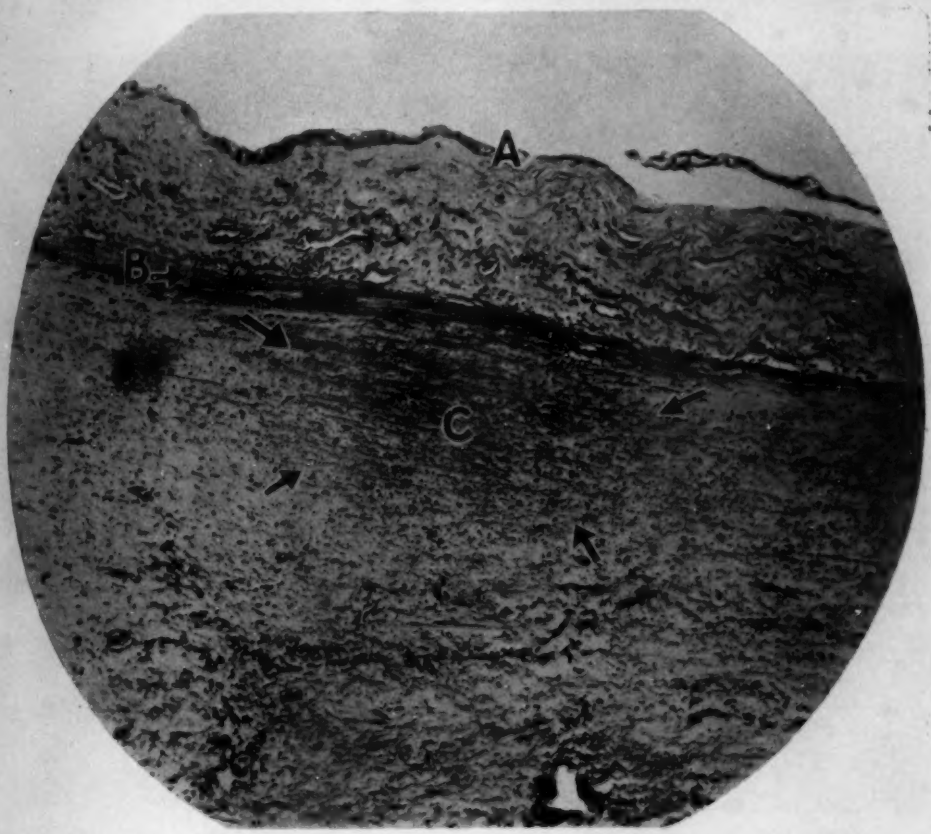

Fig. 7.—A photomicrograph of a section of the same block illustrated in figures 5 and 6 (stain, hematoxylin and eosin). In the superficial fibers of the plaque is a narrow fibrin-staining layer (*A*). At *B* there is a layer of compressed elastic tissue. At *C* is the poorly defined zone of collagenous swelling seen in figure 6.

cardial "fibrinoid" with that in the thrombus itself was not always demonstrable. In one case, the thrombus in the right auricular appendage was a recent one, although its contralateral mate exhibited the typical structure already described. The thrombi in two other cases were fresh structures.

The silver stains uniformly demonstrated argyrophilic fibers in young connective tissue. When material taking stains for fibrin was found infiltrating and separating collagenous bundles in endocardial plaques, there was no evidence of swelling or of fragmentation of such collagenous fibers. In one instance in which eosinophilic swelling of collagen was noted in the vicinity of the thrombus, the silver stain was useful in bringing out the outlines of the swollen collagen fibers

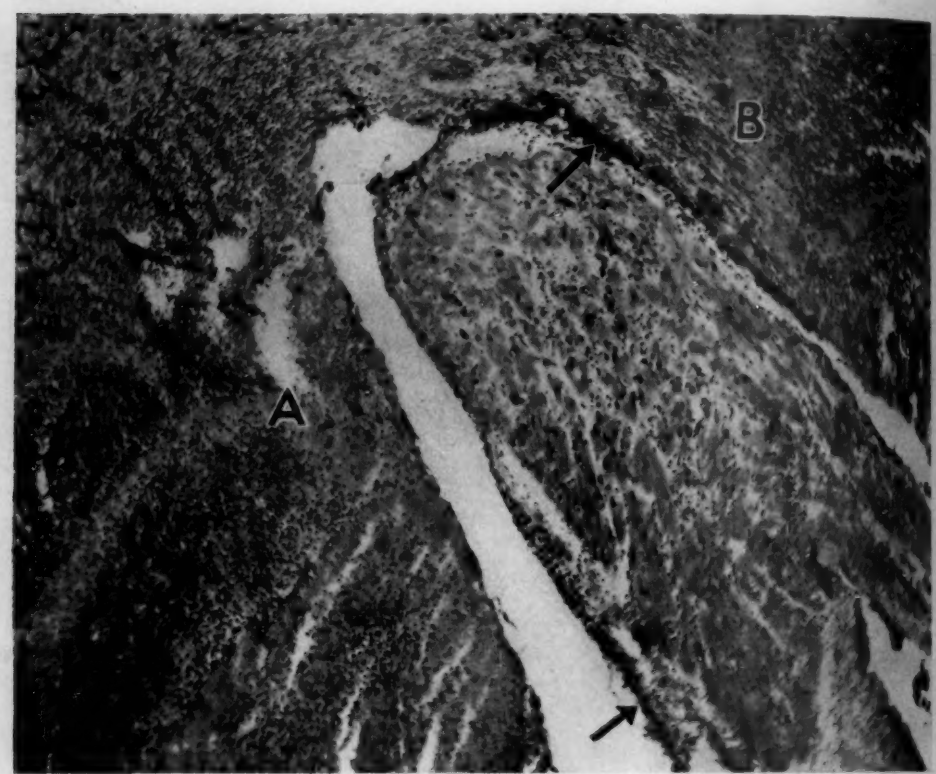

Fig. 8.—A youth 17 years old died in congestive heart failure, in a recurrence of rheumatic fever. The heart showed severe mitral stenosis, aortic stenosis and pancarditis. The photomicrograph shows a section through the left auricular appendage, in which there is fresh endocardial inflammation with necrosis (indicated in the regions marked by arrows). Fresh platelet columns are seen at *A* and *B*. This portion of the thrombus has separated after section. (Elsewhere in the section there is evidence of previous thrombus formation with organization.)

through which argyrophilic fibrils could be seen. In this instance, clear spaces were seen between swollen collagen bundles in the silver preparations, while the corresponding zones in hematoxylin and eosin stains contained eosinophilic substance.

2. Cases without Carditis: Histologically, the auricular endocardium in eight of the ten cases in which the rheumatic process was inactive revealed moderate sclerosis. Hemorrhage was uniformly absent. Calcification occurred in three of the instances. In one case, calcium was seen also in a thrombus. However, the thrombus in the right auricular appendage in this case, in contrast to that of the left side, did not contain calcium nor did the endocardium. It is important to note that in three cases (four thrombi) histologic study revealed

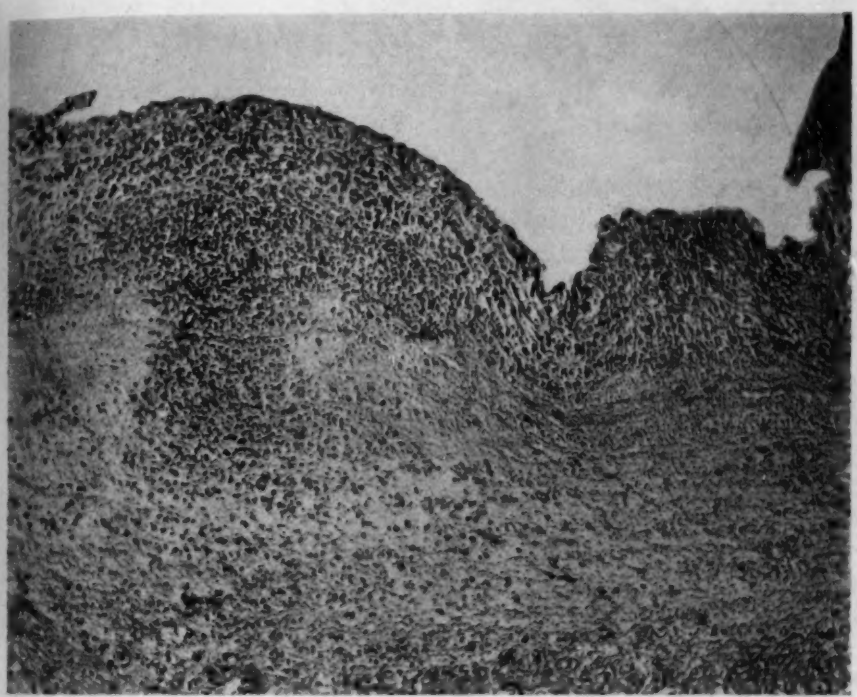

Fig. 9.—A photomicrograph of another field of the section shown in figure 8. Note the characteristic endocardial and subendocardial lesions with rows of Aschoff cells caught between lamellae of elastic and collagenous tissue. The collagen in this region was swollen, deeply eosinophilic and fuchsinophilic but did not stain like fibrin with the phosphotungstic acid-hematoxylin stain.

fibrinoid streamers and masses extending from the subjacent endocardium to the overlying laminated fibrinous thrombi.

Recent thrombus formation occurred in two cases, and in both the underlying endocardium was thin and delicate. Old, organized thrombi without any recent accretions were present in three cases. The remaining five cases exhibited progressive laminated structures essentially indistinguishable from those in the active group.

Apart from nuclear and fiber hypertrophy of a mild degree and slight fibrosis, a significant auricular myocardial lesion was noted only once in the entire group of twenty-four hearts. In this instance, a large portion of the crista terminalis of the right auricle was replaced by fibro-adipose tissue suggestive of an old infarct.

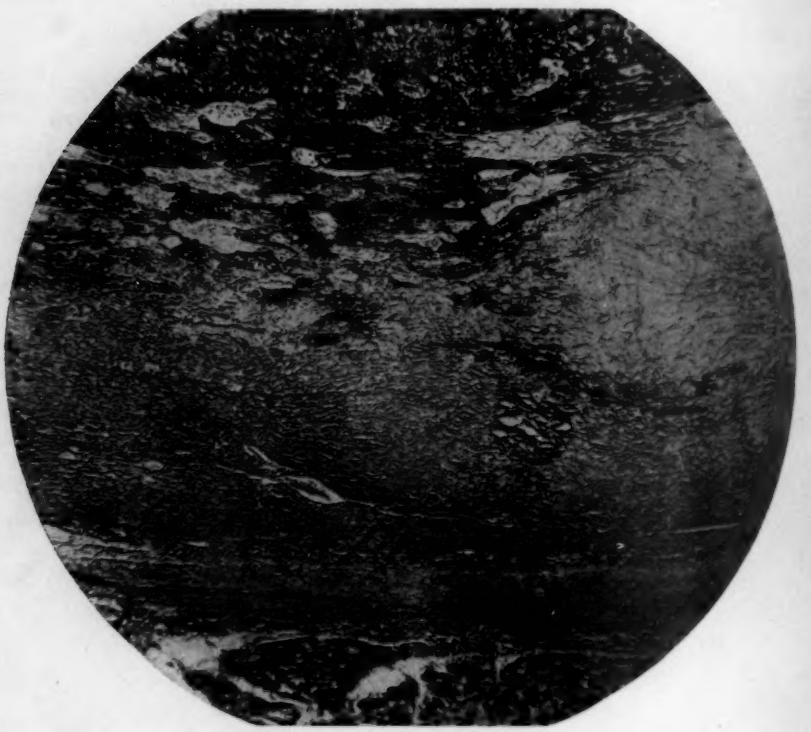

Fig. 10.—A photomicrograph of a field within the box shown in figure 2. It is stained with phosphotungstic acid-hematoxylin stain to show the intimate relation of fibrin-staining material (black) to the scar tissue of the endocardium. A little auricular muscle is seen at the bottom of the field.

CONTROLS

The controls were 157 hearts showing lesions of various etiologic types. Thirty of these presented rheumatic heart disease without auricular thrombosis. This particular group was selected only so far as it paralleled the group with thrombi in regard to age, degree of deformity of the mitral valve and presence of congestive heart failure. The controls were examined in a manner similar to that in which the other series was examined. The average age of the patients from

whom this control group of hearts came was 41 years; 75 per cent of them were in the fourth to seventh decades. Fourteen of the patients presented auricular fibrillation during life, and sixteen, sinus rhythm.

Carditis was demonstrated in fifteen of the rheumatic hearts (50 per cent). All but three had moderate to severe mitral stenosis. Auricular endocarditis was present in five (16 per cent). (Auricular appendages were not sectioned as a routine.) All showed moderate endocardial fibrous scarring, and hemorrhage and fibrinoid material were uniformly absent. Calcification occurred once.

The hearts in which rheumatic disease was inactive all showed moderate endocardial sclerosis, and one contained calcium. Hemorrhage and fibrinoid material, again, were conspicuously absent. The hearts from the patients who had had auricular fibrillation showed a severe degree of mitral stenosis.

Congestive heart failure was the cause of death in twenty of the patients, and one other gave a history of cardiac failure in the past.

Thirteen selected hearts containing auricular thrombi from patients with hypertensive and arteriosclerotic heart disease who died in cardiac failure were also examined. These patients averaged 56 years in age. Eight of them had sinus rhythm, and five, auricular fibrillation. A thrombus occurred in the left side alone in one heart, in the right side alone in ten and bilaterally in two. The thrombi were mainly recent structures with varying degrees of organization. The only constant auricular change was mild endocardial sclerosis. Cellular proliferation and fibrinoid material were uniformly absent from the underlying endocardium. An underlying auricular myocardial infarct was found once.

A gross examination of thirty-four unselected hearts from patients with hypertensive arteriosclerotic heart disease with coronary thrombosis revealed no significant lesion in the auricles or their appendages. Twenty-two of the thirty-four patients died in congestive heart failure.

A control group of 80 unselected hearts from patients with syphilitic aortitis was examined. Twenty of these patients died in heart failure as a result of their cardiovascular lesions. Aortic insufficiency was present in twenty-five. The auricles were uniformly without thrombosis except two which had recent thrombi in their right auricular appendages.

COMMENT

Although an extensive literature on thrombosis and rheumatic heart disease exists, no detailed correlation of the type reported here could be found. Harvey and Levine,[14] analyzing 111 cases of uninfected mural thrombi in all types of heart disease, found the most common sites of thrombosis to be the tip of the left ventricle and the right

14. Harvey, E. A., and Levine, S. A.: Am. J. M. Sc. **180**:365, 1930.

auricular appendage. When the cases of auricular thrombi in their entire group were analyzed, it was found that the right side was involved more frequently than the left. However, left auricular thrombosis was preponderant in their eighteen cases of chronic valvular (presumably rheumatic) disease. They also found a greater incidence of auricular thrombosis in cases of auricular fibrillation.

In attempting to evaluate the factors which may promote intracardiac thrombosis, the alteration in the hemodynamics consequent to mitral stenosis and auricular fibrillation seems, at first glance, important. Since in all twenty-four cases in this study there was either a definite history of congestive heart failure or signs of heart failure at autopsy, it appears that this factor may also contribute to the production of auricular thrombi.

Nevertheless, these factors must not be given too much weight in considering the pathogenesis of auricular thrombosis in rheumatic heart disease, for in the entire series of rheumatic hearts collected in this laboratory during the past fifteen years the incidence of auricular thrombosis is only 13 per cent, while the incidence of mitral stenosis is 87 per cent, the incidence of auricular fibrillation is 39 per cent, and the incidence of congestive heart failure is 70 per cent. Since these figures clearly show that intracardiac thrombosis is a relatively uncommon event in rheumatic heart disease, attention must be directed to some other factors.

Endocardial lesions, like circulatory changes, may lead independently to thrombosis. When the two disturbances are associated, it is difficult, if not impossible, to determine which played the major rôle in the formation of the thrombi. The high incidence of endocardial scarring in the hearts with thrombi seems significant and recalls the intimal changes in arteries and veins which so frequently furnish the soil for thrombi. However, the endocardial scarring in these hearts, like mitral stenosis, is probably not greater than in the hearts without thrombi. Gross demonstrated microscopic lesions of the left auricular endocardium in 100 per cent of his cases of active rheumatic heart disease, and he believed that the scarring found in hearts showing old rheumatic processes may be attributed to previous activity. Böger [15] suggested that the diffuse type of endocardial sclerosis may represent the response of the elastica to dilatation and stretching.

Active auricular inflammation was found beneath the thrombus in the left auricle in two cases, in the left auricular appendage in five and in both in three, a total of ten cases (42 per cent). In one instance a macroscopic thrombus was found over active auricular endocarditis of the type described by MacCallum and Von Glahn. The differen-

15. Böger, Alfred: Beitr. z. path. Anat. u. z. allg. Path. **81**:441, 1929.

tiation of the inflammatory process from the nonspecific process associated with organization of the thrombus was not always easy, though demonstration of similar inflammatory changes in the endocardium nearby was a helpful guide.

To determine whether or not the finding of activity anywhere in the heart was common in cases of auricular thrombosis, the total incidence of carditis in this group was compared with that in a group of thirty control cases. As noted before, the controls were selected to parallel closely the cases with auricular thrombi in all other respects, i. e., age, sex and cardiac defects. In this control group, the total incidence of activity was 50 per cent, and in the thrombotic group it was 58 per cent. However, active auricular endocarditis was present in ten cases (42 per cent) with thrombi and in only five (16 per cent) of the rheumatic controls. In order to keep the factor of congestive heart failure equal, the cases in both groups in which death occurred from heart failure were taken separately and analyzed for activity. In the group of twenty-one with thrombosis (death from heart failure) thirteen (62 per cent) showed carditis; of these, ten (47 per cent) showed active auriculitis beneath the thrombus. The rheumatic control group exhibited carditis in thirteen (62 per cent) of the twenty-one cases in which death occurred from heart failure; of these, only five (24 per cent) showed active auriculitis. *Thus, when the factors of congestive heart failure and active carditis are controlled, active auricular endocarditis is found in 47 per cent of the cases of rheumatic heart disease with auricular thrombosis in contrast to 24 per cent of the cases of a similar group without thrombosis.* This finding is made more significant by the fact that the incidence of auricular fibrillation and the degree of mitral stenosis in both groups are practically the same.[16] Furthermore, the whole group of thrombi includes fresh thrombi of the right side not related to underlying activity. Weiss and Davis[17] made an analysis of the records of 164 cases of fatal rheumatic heart disease. Twenty-eight of these cases showed auricular thrombi. The data revealed an incidence of active inflammation in 18 per cent of the cases with thrombi as contrasted with 47 per cent in the entire series. Although these authors believed that activity of the rheumatic process was not an important factor in auricular thrombosis, the difference between their results and those reported here may be due to the fact that the present findings were obtained by histologic examination of the original material. Why thrombosis

16. In thirteen of the twenty-one cases with auricular thrombosis in which there was congestive heart failure there was auricular fibrillation, and eleven of the twenty-one cases without thrombosis showed this arrhythmia.

17. Weiss, Soma, and Davis, David: Am. Heart J. **9**:45, 1933.

occurs in some patients and not in others is impossible to determine in all cases. The evidence gathered thus far indicates that continued auricular activity probably plays an important rôle in thrombosis. Whether the old organized thrombi beneath which no recent endocarditis could be found had their origin at a time when there was activity in the subjacent endocardium cannot be established by direct evidence.

Further support for this opinion is given by the finding of definite recent or old endocarditis of the rheumatic type beneath the majority of the thrombi in the left auricular appendages. Incidentally, this relationship has not been noted hitherto nor, indeed, has the occurrence of typical auriculitis in this portion of the heart been described previously.

It is worthy of note that in this series right auricular thrombi resembled those found in other forms of organic heart disease. Even in cases with bilateral thrombi, the structures on the right side were relatively fresh (with one exception), were not located on zones of inflammation and were found only in the auricular appendage. In four of five instances with thrombosis limited to the right side, congestive heart failure was present without evidence of local auricular activity. (In the fifth there was advanced carcinomatosis.) The inference seems reasonable that in the right auricle thrombus formation is related to disturbances in hemodynamics.

Because of the contention of Klinge and his co-workers that fibrinoid material occurring in supporting tissue, valves and the walls of vessels specifically represents altered collagen and that it denotes the rheumatic (or so-called hyperergic) tissue reaction, special attention was paid to the distribution of this eosinophilic material and its relation to adjacent structures. It is noteworthy that Klinge did not differentiate fibrinoid swelling of collagen in truly interstitial tissue (e. g., intermuscular supporting tissue) and in special mesenchymal structures found in the vascular system (valves, subendocardium and vessels) from fibrinous material found in the same situations. In this connection, it is interesting to recall that Von Glahn in his description of the auricular lesions in rheumatic fever clearly described fibrin on the surface and in the subendocardial region, together with swollen, degenerated collagen. He was careful to note that the latter stained deeply with fuchsin. Gross, who noted microscopic left auricular lesions in 100 per cent of his cases of active rheumatic heart disease, referred only to eosinophilic swelling of collagen, without comparing it with deposited fibrin. Since both collagen and fibrin stain intensely with eosin, it is essential to differentiate them by all available means. In three instances in this series of cases of rheumatic heart disease with auricular thrombosis, swollen eosinophilic collagen (fibrinoid in appear-

ance) was encountered in the subendocardium. On differential staining with Mallory's phosphotungstic acid-hematoxylin stain and by Van Gieson's method, these swollen bands retained the staining property of collagen, while in their vicinity, in the same section, overlying fibrinous thrombi showed characteristic fibrin-staining reactions.

The observations of Clark, Graef and Chasis [2] on so-called fibrinoid lesions in the coronary arteries and in arteriosclerotic and syphilitic aortas led to the conclusion that the fibrinoid material represents compressed and hyalinized elements of the blood (chiefly fibrin) and that the subsurface location of such material is due to partial organization of deposits originally on the surface. They found no evidence to support the contention that fibrinoid material represents altered collagen.

The same impression was gained from the present study, namely, that fibrinoid material in the thrombus and endocardium is derived from altered elements of blood and that the growth of endocardial plaques may be enhanced by the deposit of flat parietal thrombi that undergo organization. The case of verrucous auricular endocarditis illustrated the relationship of what might be termed fibrinoid swelling of the subendocardial tissue to thrombus formation. Tinctorially, the fibrinoid material in the endocardium was identical with that in the overlying thrombus and, indeed, fused with it through minute streamers. Other cases in the series showed, too, in random as well as in serial sections, bands stainable with eosin and trinitrophenol extending from the thrombus into the depths of the endocardium. It seems more reasonable, in the presence of obvious endocardial and subendocardial changes, to acknowledge an alteration in permeability or even microscopic fractures of the surface layer permitting infiltration of elements of the blood, particularly fibrin or fibrinogen, into the tissue spaces of the subendocardium.[18] In the scarred inactive endocardium progressive thrombosis with compression and lamination may account for the location of the fibrinoid material. *Furthermore, the histologic identity of the fibrinoid material found in cases of active disease with that found in cases of inactive disease supports the view that this material when found in intimate relation to the blood stream truly represents fibrin formation.* The fibrinoid material herein noted is not to be identified with the fragmented deeply eosinophilic collagen of the active Aschoff body.[19] The observation that "fibrinoide Verquellung des Grundsubstanz"

18. This view is similar to that advanced by P. Schürmann and H. E. McMahon (Virchows Arch. f. path. Anat. **291**:47, 1933) in explaining the fibrinoid appearance of the arteriolar lesions in cases of malignant nephrosclerosis, suggesting the imbibition of plasma containing fibrinogen.

19. Gross in his studies on the Aschoff body noted that the altered collagen practically never shows affinity for trinitrophenol, and we can support his observations with our own.

of the type described by Klinge occurs in many interstitial tissues is not questioned. In fact, fibrinoid swelling of collagen was observed in the subendocardium in three instances in this series. In one it was intimately associated with thrombus formation, and in another it was seen deep in a section of the endocardium on which a hyalinized thrombus had been formed.

In view of the overlying endothelial changes [20] occurring in the active stages of rheumatic endocarditis, it seems more reasonable to attribute the occurrence of fibrinoid material to a deposit of elements of the blood, particularly fibrin. When deeply staining swollen collagen lies side by side with equally deeply staining compressed fibrin, ordinary staining may give them an identical appearance. We have found Mallory's phosphotungstic acid-hematoxylin stain to be the only one consistently of aid in differentiating these substances.

In old scarred auricles the thrombi encountered are similar to those seen on scarred plaques in other vascular channels. It is interesting to see how the organization of a small parietal thrombus may lead to an increase in thickness of the endocardial plaque, a phenomenon which recalls a similar process in arteriosclerosis.

SUMMARY

Twenty-four (13 per cent) of 178 hearts showing rheumatic disease also presented auricular thrombosis. The average age of the patients from whom these twenty-four hearts were obtained was 47 years. Disorders of rhythm had been encountered in two thirds of the group, the majority having persistent auricular fibrillation. Congestive heart failure was present at autopsy in twenty-one of the patients. Severe mitral stenosis was found in eighteen hearts of the twenty-four.

Macroscopic auricular scarring was conspicuous in twenty-two of the twenty-four hearts. In fourteen, thrombosis was confined to the left auricle, in five to the right, and in five, there was bilateral thrombosis. Thrombus formation occurred over a MacCallum patch in five hearts.

Evidences of carditis were present in fourteen of the twenty-four specimens. Of these, ten showed active inflammation in the auricular endocardium beneath the thrombus.

20. In his studies, Von Glahn [1b] described a striking palisade arrangement of Aschoff cells along bands of hyaline material identified as swollen collagen. Further, he wrote: "In the acute stages there is considerable edema, and fibrin is found in varying amounts among the distorted cells, but none can be demonstrated in the homogeneous material about which the Aschoff cells are gathered. Fibrin is readily demonstrated in places upon the surface of the endocardium over those areas where the endothelium has disappeared, and is also present in considerable quantity in the edematous vascular zone near the myocardium." He also described the inclusion of fibrin in the organizing process.

In seven hearts a thrombus of the left auricular appendage was associated with fresh and recent auriculitis like that seen elsewhere in the left auricle in rheumatic fever.

In hearts in which the rheumatic process was inactive, the thrombi exhibited gross and microscopic characteristics similar to those of thrombi in hearts in which the condition was active.

Among patients who died of rheumatic heart disease in congestive failure, active auricular endocarditis was observed twice as frequently in those with auricular thrombosis as in those without this, although the incidence of auricular fibrillation and the degree of mitral stenosis were about the same in both groups.

In twenty-two thrombi, organization was demonstrated at the bases and accounted in part for the inclusion of fibrin-staining material in the endocardial plaques.

CONCLUSION

Certain conditions appear to favor the development of auricular thrombi. These are severe mitral stenosis, together with congestive heart failure, auricular fibrillation and continued local inflammation. On analysis of these factors, the persistence of active inflammation appears to be the chief one in the light of present knowledge.

The occurrence of fibrin-staining material beneath the endocardium can be differentiated from the focal swelling of collagen in the same structure by the use of Mallory's phosphotungstic acid-hematoxylin stain. The fibrin-staining material thus demonstrated is presumably derived from the proximate blood stream.

NOTE.—Although the clinical value of a study of auricular thrombosis would be enhanced by a consideration of embolic lesions, this was not included because limitations on the autopsies often prevented complete examinations, and the difficulty in distinguishing old vascular lesions due to autochthonous thrombi from organizing emboli precluded accurate diagnosis in too many instances.

Laboratory Methods and Technical Notes

PRACTICAL ILLUMINATED SHELF FOR THE PATHOLOGIC MUSEUM

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Effective display of preserved pathologic material is essential for the correlation of clinical, gross and histologic aspects of disease.

In 1926 the late Dr. E. R. LeCount and I experimented with an inexpensive shelf capable of holding an adequate display of specimens for demonstration. As a result, a shelf 56 inches (142 cm.) from the floor (eye level for the majority of students) was agreed on. It was made of seasoned maple 1 inch (2.5 cm.) thick, 10 inches (25.5 cm.) wide and 40 feet (12 meters) long, backed by a 4 inch (10 cm.) strip ½ inch (1.2 cm.) thick, to prevent dislodgment of the specimens. It was supported by steel angle irons ½ inch (1.2 cm.) thick, 2 inches (5 cm.) wide and 10 inches (25.5 cm.) long, bolted to the ends of museum cases directly behind it. These heavy supports were necessary, as many specimens in jars filled with preservative weigh as much as 40 pounds (18 Kg.), and as many as 70 small or 40 large specimens are used for each display. The wood was treated with acid-proof stain and two coats of linseed oil. Cards requesting that there be no handling of the labels or jars were displayed above the shelf. Cards 4 by 6 inches (10 by 15 cm.), thumb-tacked to the front edge of the shelf, labeled the specimens.

The specimens were oriented to demonstrate the featured aberrations, so that no handling by students was necessary. This precaution prevented the disorientation common when a group handles a series of objects and preserved the proper demonstration for succeeding observers. It also prevented smudging of the glass by finger marks and roiling of the preservative. Breakage, loosening of covers and loss of catalog tags were reduced to a minimum, although the edge of the shelf soon became shredded from the repeated use of thumb-tacks. Cork or heavy felt strips along the edge of the shelf might have prevented some of this disintegration.

One great disadvantage in using this shelf was the poor illumination. Overhead ceiling lights were inadequate during the winter months. At all times, students blocked the light from the windows. I remedied these defects by using footlights as follows. Thirty-two porcelain-enameled steel reflectors (fig. 1 *B*) 9 inches (23 cm.) long by 2⅜ inches (6 cm.) high, were placed in a trough 2½ inches (about 6 cm.) wide and fastened to the front edge of the original shelf. The wiring was arranged so that alternate lights, i. e., 1, 3, 5 or 2, 4, 6, could be operated by a separate switch or all lights at once by both switches. Each reflector had a 25 watt tubular lamp.

To provide a place for descriptive cards, a 4 inch (10 cm.) strip of wood was employed as the front edge of the reflector trough (fig. 1 *A*). To this a right-

From the Norman Bridge Pathological Laboratory, Rush Medical College, University of Chicago.

angled strip of 26 gage tin was nailed at the top and bottom edges to provide a continuous groove, into which cards were slipped and easily moved to center beneath the specimens on the shelf. This method of handling the cards had several advantages over the former method. Cards were not lost by working loose from

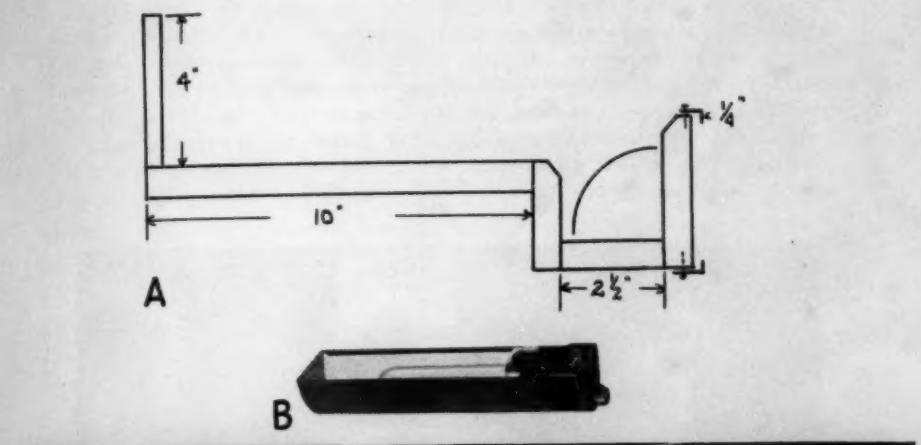

Fig. 1.—Museum shelf: *A*, cross-section view of the shelf, trough for reflectors and card rail. *B*, one of the reflectors used in parallel, 16 per circuit. *C*, a general view of the shelf with a display of pathologic specimens.

the thumb-tacks, nor were they marred by repeated punctures from the tacks. Photomicrographs or other illustrations were slipped into these grooves without injury.

A white glass or cardboard behind some of the large specimens eliminated some of the high lights, especially from round jars. The white also illuminated

some thin tissues posteriorly, such as mesentery, translucent tumors and cysts. Using alternate lights for some of the large specimens accentuated hollows and protuberances. Such lighting was also helpful when jars were set at wide distances, as was necessary when students of pathology were given practical examinations or on bright days.

About 250 students per week, in groups of from 30 to 40, viewed the specimens displayed on this shelf. The exhibits were designed especially to aid students taking courses in pathology. Faithful attendance, however, assured all students an opportunity to examine about 2,000 specimens in a year; few failed to appreciate such an advantage. An outline given each new group of students frankly urged them to cultivate their vision by inhibiting their desire to handle jars.

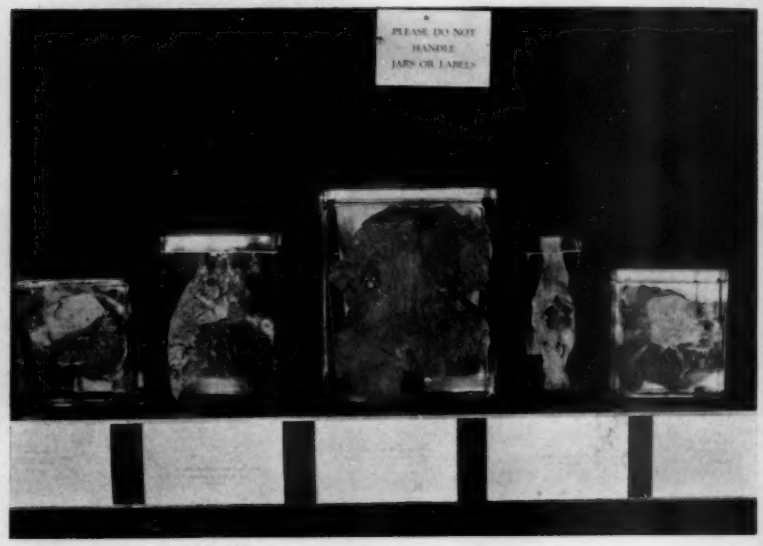

Fig. 2.—A close-up view of a portion of the shelf with the lights turned on full, illustrating the lack of annoying high lights.

This led to a friendly competition among members of the class to determine who had the most retentive vision. The cards, bearing typewritten data to within one-fourth inch of the top and bottom edges, usually contained brief clinical notes, pertinent autopsy observations, histologic observations and references to literature for further study or to which these specimens had contributed. The references stimulated reading, while the clinical notes correlated the symptoms and pathologic changes.

SUMMARY

A simple sturdy shelf for the display of museum preparations is described, effective illumination of which with a minimum of glare is provided by reflectors arranged as footlights. The tin card rail allows a rapid assembling and easy lateral adjustment of cards.

Case Reports

CORONARY ANEURYSMS WITH THROMBOSIS IN RHEUMATIC CARDITIS

Unusual Occurrence Accompanied by Hyperleukocytosis in a Child

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Although inflammatory involvement of the coronary arteries in rheumatic fever is common, the damage is seldom as severe as that encountered in the case to be reported. Coronary lesions accompany the acute myocardial state regularly (Karsner and Bayless [1]); the main coronaries are inconstantly involved (Gross, Kugel and Epstein; [2] Von Glahn and Pappenheimer [3]) and the smaller branches invariably (Klotz [4]). The intensity and extent of the lesions vary greatly. Intimal lesions vary from slight subendothelial thickening to intense endothelial proliferation, polymorphonuclear infiltration, severe destruction of the elastica, with narrowing of the lumen, and occasionally thrombosis. The media shows lesions ranging from edema to profuse cellular infiltration, degeneration and necrosis. In the adventitia, infiltration by inflammatory cells of various types is the common picture. The existence of Aschoff bodies in the adventitia and media, which is the main, if not the only point of specificity in rheumatic fever, is a matter of debate among various authors. The ultimate result of the various acute alterations is fibrosis and premature coronary sclerosis.

REPORT OF CASE

A boy of 2 years and 9 months with complaints which dated back one month was admitted to the hospital Sept. 17, 1935. During the period of illness he had been pale, listless and lacking in energy and had had four or five large foul-smelling green stools daily. Ten days before admission he had been put to bed because of a fever and what his mother described as "a reddish rash and strawberry tongue." Following this stage he complained of tenderness in his bones and joints when handled. For two days he had had a slight cough. Further questioning revealed that he had always been a pale weak baby and for the last nine or ten months had had occasional "blue spells."

On admission the child appeared quite ill. The submaxillary and right axillary lymph nodes were slightly enlarged and the inguinal group barely palpable. The conjunctival surfaces showed injection. The throat was congested, the tonsils moderately enlarged and the nose discharging. The chest was normal. The

From the Department of Pathology, Hospital for Sick Children.

1. Karsner, H. T., and Bayless, F.: Am. Heart J. **9**:557, 1934.
2. Gross, L.; Kugel, M. A., and Epstein, E. Z.: Am. J. Path. **11**:253, 1935.
3. Von Glahn, W. C., and Pappenheimer, A. M.: Am. J. Path. **2**:235, 1926.
4. Klotz, O.: Tr. A. Am. Physicians **27**:181, 1912.

cardiac rate was from 132 to 136 beats per minute. The heart was of normal size, and the sounds were of fair quality and clear. The abdomen was soft and not tender; the liver was palpable 1½ fingerbreadths below the right costal margin; the tip of the spleen was just palpable. The temperature was gradually reduced from 103.4 F. to normal in ten days. The red blood cell count was 4,200,000; the hemoglobin was 60 per cent; the white cell count was 45,000; the differential count gave percentages as follows: polymorphonuclears 88, myelocytes 11 and lymphocytes 1. Platelets were plentiful. Later the white cell count rose to 76,000, with 98 per cent polymorphonuclears. The blood culture was negative. No hemorrhages of the skin or of mucous membranes were found. The nasopharyngitis cleared, and the temperature returned to normal, but the child died suddenly on his tenth day in the hospital. The high leukocyte count with an excess of polymorphonuclear cells, including immature forms, and the palpable lymph nodes and spleen led to a clinical diagnosis of acute myeloid leukemia. The diagnosis was further supported by the pains in the joints—a symptom that frequently occurs in the early stage of leukemia.

Autopsy.—The autopsy was made soon after death. The body was normally developed but was slightly undernourished. The skin and mucous surfaces were unusually pale. The lymph nodes in the neck and groin were palpable but were soft and movable. The abdominal wall and omentum contained very little fat. The peritoneal surfaces were smooth and glistening, and the cavity was free from abnormal contents. The mesenteric lymph nodes were moderately enlarged, fairly firm and pink. The spleen contained a large area of recent infarction. The pleural surfaces on both sides were smooth and shiny. The lungs were crepitant, pale and soft throughout. The peribronchial lymph nodes presented nothing unusual.

In the heart many interesting changes were found. The visceral and parietal layers of the pericardium were glued together by a thick fibrinous exudate which was undergoing organization, obliterating the pericardial cavity. When the two layers were torn apart, a shaggy rough grayish red surface presented, on which the landmarks of the heart were largely obscured. The exudate was fairly uniform over the whole heart and measured up to 3 mm. in thickness. The parietal pericardium was greatly thickened and lined by a similar rough surface. A culture of this exudate proved sterile. The heart was slightly enlarged and weighed 92 Gm., but the enlargement was due mainly to dilatation of the ventricles. The chambers of the heart contained postmortem blood clot but no antemortem thrombus. A prominent rounded swelling about the size of a marble lay in the anterior wall of the right ventricle, near the base, in the angle between the origin of the aorta and the pulmonary artery, and protruded on the pericardial and endocardial surfaces. This nodule was soft and pale grayish white in contrast to the adjacent reddish myocardium. The mural endocardium lining the left auricle was rather thick, opaque and wrinkled. The valve leaflets and cusps were thin, regular and semitranslucent. No gross evidence of endocardial roughening or of implantation of vegetations was found on any of the valves. The musculature was grayish red and slightly hypertrophied, the left ventricle measuring 0.9 cm. and the right 0.4 cm. in thickness. There was no evidence of softening or scarring in the wall.

The coronary arteries took their origin in the usual position, and the apertures were normal in size and shape, as were the vessels in the first 3 mm. of their course. Beyond that point, both the left and the right artery expanded abruptly into large aneurysmal dilatations. The first part of the aneurysm of the left artery was round or globular, measuring 1.2 cm. in diameter. This portion was

limited by a constriction, beyond which the descending branch again ballooned out and tapered gradually to reach the normal caliber of the artery about 3 cm. from its origin. The circumflex branch opened from the first aneurysmal dilatation, was normal in size and continued in its normal direction.

The right coronary aneurysm consisted of a large irregular sac, which measured 1.5 cm. across its base and 2 cm. in length. The cavity was entirely plugged with a mass of friable grayish red thrombus, which appeared slightly laminated in character. Beyond the thrombus, the vessel contained dark red postmortem blood clot. It was this massive thrombus which was responsible for the swelling on the anterior surface of the heart. In the thought that this nodule might be an

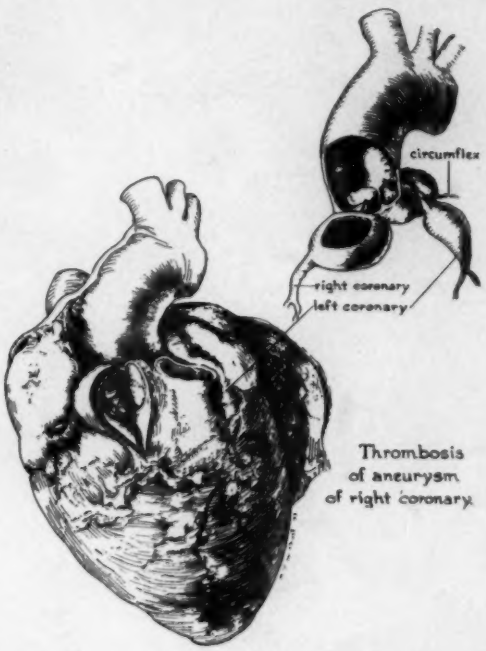

Fig. 1.—Drawing of the heart showing the aneurysm of the right coronary artery, with thrombosis (opened), and the aneurysm of the left coronary artery, which was patent. The pericardium is thick and shaggy.

abscess, it was carefully opened and cultured, but it proved to be sterile. There was no gross evidence of myocardial infarction resulting from the coronary occlusion.

Microscopic Examination.—In the myocardium was found an intense diffuse inflammatory reaction of rheumatic character. Moderate perivascular edema and fibroblastic proliferation were seen about the smaller arterial branches. In these perivascular areas were groups of characteristic Aschoff cells, some well defined, others irregular in outline, with prominent vesicular nuclei and abundant pale basophilic cytoplasm. Lymphocytes and polymorphonuclear leukocytes were also present in moderate numbers. The smaller arteries themselves, however, showed

no intrinsic change. Apart from the perivascular lesions, the interstitial tissue of the muscle was infiltrated by polymorphonuclear neutrophils in profusion, and among the leukocytes were also scattered Aschoff cells. Small groups of muscle fibers in these areas showed early degenerative change. The latter reaction was

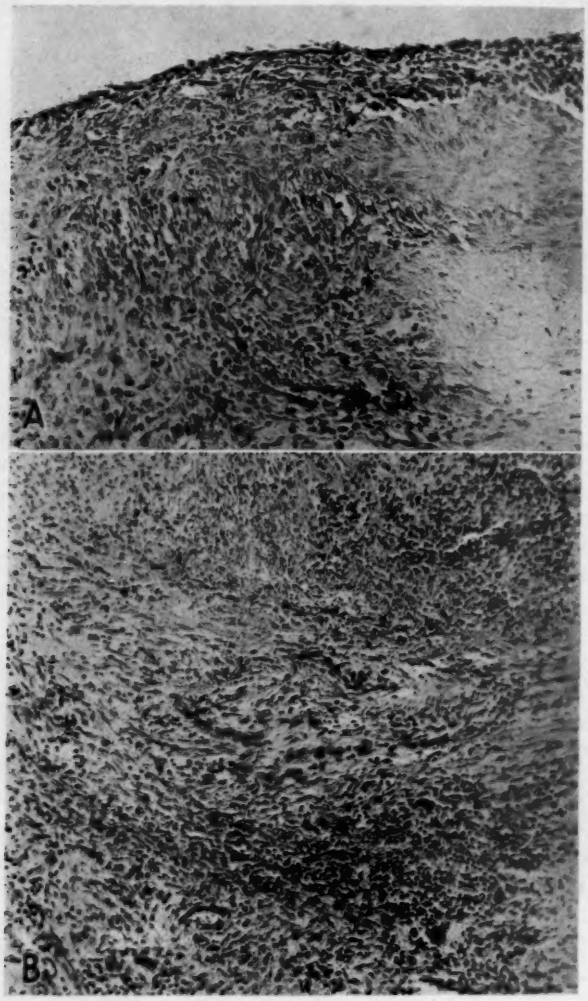

Fig. 2.—*A*, inflammatory reaction with degeneration of the media in the left coronary artery; × 120. *B*, intense inflammatory reaction in the right coronary artery; × 120.

confined almost entirely to the musculature of the left ventricle. The right ventricle and the auricles presented occasional perivascular Aschoff nodules but no reaction of the diffuse exudative type. No evidence of recent or old infarction of muscle was found.

On the pericardial surface the lesion was chiefly represented by an exudate undergoing organization, with, also, less prominent but characteristic cellular rheumatic lesions. Three distinct layers could be recognized. Adjacent to the

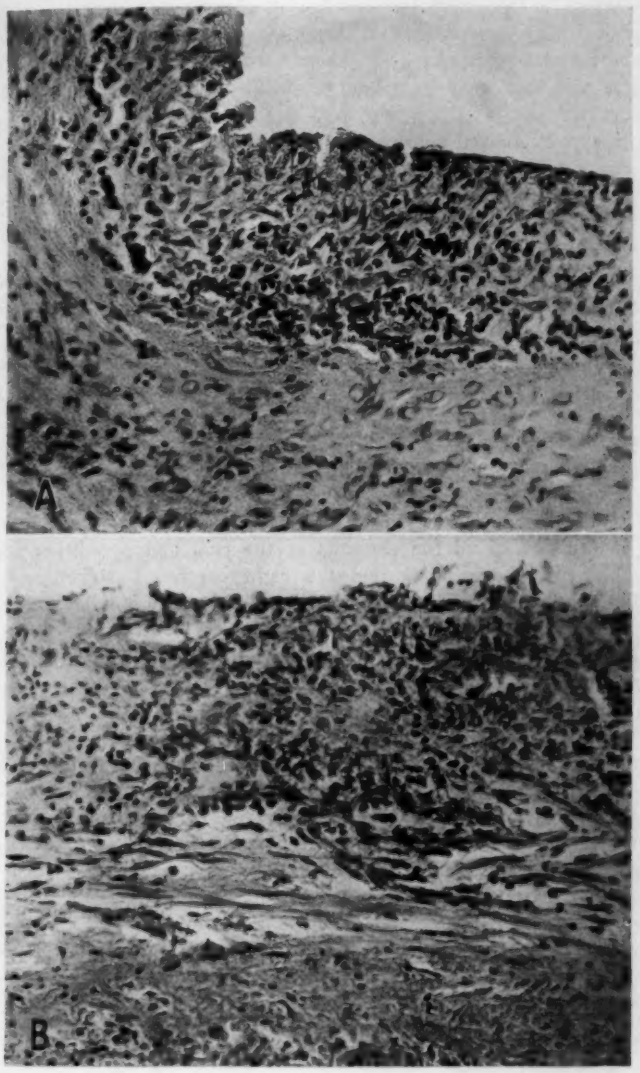

Fig. 3.—*A*, intense intimal thickening with cellular proliferation and infiltration in the left coronary artery; × 200. *B*, wall of the aneurysm of the right coronary artery, composed of a few fibrous strands with a thick pericardial exudate (above); the thrombus in the lumen (below); × 200.

myocardium were large numbers of inflammatory cells, including polymorphonuclear leukocytes, plasma cells and lymphocytes. These cells infiltrated the subepicardial fat, surrounded the blood vessels and accompanied them as they

branched abruptly into the muscle. Many Aschoff cells were present in small groups. Superficial to this layer was a thick zone of edematous granulation tissue, with proliferating fibroblasts and new capillaries invading the fibrinous exudate. Inflammatory cells and Aschoff cells were found in moderate numbers. On the surface were large accumulations of fibrin without inflammatory cells. The parietal pericardium showed similar strata of exudate. In many areas on both visceral and parietal pericardium the exudate had been entirely vascularized, and it is possible that there may have been established a capillary connection between the two layers.

The left auricle showed a prominent subendocardial thickening of hyaline and collagenous character, with numerous scattered mononuclear cells palisaded along the more superficial layers. The muscular part of the wall also presented a sprinkling of cells, with occasional Aschoff bodies.

The mitral valve was regular in outline and was slightly thickened and edematous. A sprinkling of mononuclear cells lay along the surface, with here and there a definite attempt at palisading. The endocardial surface was intact, and no deposits of fibrin or of thrombus were present.

In the aorta perivascular collections of cells were present about the blood vessels of the outer third of the media. Vascularization had not extended beyond the outer third nor was there scarring of the media. The cells included large and small mononuclear varieties and an occasional polymorphonuclear type. The adventitia presented large numbers of various inflammatory cells about the vasa vasorum, but no Aschoff nodules.

The coronary arteries suffered an exudative necrotizing reaction leading to extensive destruction of all the elements of the wall and permitting aneurysmal dilatation. The left descending and the right coronary artery were severely affected, but the left circumflex branch, only slightly. Lesions were most intense in the proximal part, diminishing with the caliber of the vessel.

Intimal thickening of various degrees was conspicuous. The mildest form was shown as a subendothelial hyaline deposit. Again a cellular layer was seen, the result of endothelial proliferation. The most extreme thickening, however, resulted from a combination of these, together with the presence of large numbers of mononuclear inflammatory cells. The adventitia contained quantities of fibrin, and the fat and fibrous tissue were invaded by cells. The media was edematous, and the individual muscle cells were swollen and pale. The muscle layer was broken frequently by inflammatory or granulation tissue. The internal elastic layer became frayed and broken, leaving only small isolated fragments. Cellular infiltration was marked and consisted of large numbers of polymorphonuclear cells, plasma cells and lymphocytes and scattered groups of large basophilic cells, suggestive of Aschoff cells. The latter cells were confined to the media and adventitia, while the intima disclosed chiefly a mononuclear variety. These lesions produced complete disintegration of the muscle and elastic tissue of the media. Near the base of the involved arteries, the destruction of tissue was so extensive that a large part of the aneurysmal wall was maintained by only a thin layer of fibrous tissue. This was most clearly portrayed in the right coronary aneurysm, in which only a few fibrous strands and a layer of inflammatory exudate covered the thrombus which filled the lumen. The thrombus was of recent origin, showed no attempt at organization and therefore afforded little protection to the weakened arterial wall. The left coronary artery was freely patent throughout. The absence of infarction in the right ventricle may have been due to the thrombosis being incomplete and a partial lumen remaining, or the capillary bed

being established from the parietal pericardium through the organized pericardial exudate may have maintained adequate circulation.

The other organs were carefully searched for evidence of inflammation affecting the peripheral blood vessels, particularly the lungs, but they were found to be significantly free. The splenic infarct may have been an embolic occlusion from a thrombus dislodged from the mouth of the right coronary artery and carried in the systemic circulation.

The bone marrow showed marked myeloblastic activity, but infiltration of organs by myeloblastic cells as in myeloid leukemia was not evident.

COMMENT

The evidence of the rheumatic origin of the coronary lesions lies in the distinctive characters of the associated myocardial, auricular and pericardial lesions, with definite Aschoff bodies, the auricular thickening and cellular reaction, the aortic lesions and the microscopic valvulitis. The arterial lesions included: intimal thickening of hyaline nature, cell proliferation and infiltration; medial edema, degeneration and replacement by granulation tissue, intense cellular reaction, including groups of Aschoff cells, and fragmentation of the internal elastic membrane; adventitial edema and inflammatory cell reaction. The peculiar histologic features of rheumatic disease of the coronary arteries have a specific character which distinguishes such disease from other types of vascular disease. Periarteritis nodosa presents a more generalized vascular involvement, giving rise to symptoms simulating a variety of diseases, as acute abdominal and gastro-intestinal conditions, renal disease, polyneuritis and polymyositis, as well as cardiac and pulmonary disease. The pathologic picture is a typical ringlike necrotic process in the media of the muscular or visceral arteries (medium-sized vessels), with arteritis also in the adventitia and in the intima secondarily. Eosinophils and plasma cells are numerous. The formation of miliary nodules and aneurysms is common in periarteritis nodosa, and thrombosis is frequent.

The frequent association of rheumatic fever and periarteritis nodosa has been stressed by Friedberg and Gross,[5] who suggested that rheumatic fever is the basis of the widespread vascular alterations in the viscera known as periarteritis nodosa.

The vascular lesions found in general infectious diseases may be degenerative, inflammatory or proliferative, and the involvement is fairly general. The histologic picture does not differ essentially from that of rheumatic fever, except for the absence of Aschoff bodies (Karsner and Bayless [1]).

Suppurative inflammations by direct extension may lead to acute arteritis of part or all of the arterial wall. That a suppurative mycotic process from the pericarditis existed in the case reported here is ruled out by the fact that the cultures of the pericardial exudate were sterile.

The absence of gross lesions in the mitral valve may be worthy of comment. A thrombotic and vegetative process on the cardiac valves is very rare in young children (de Vecchi,[6] White,[7] Holt), and when it

5. Friedberg, C. K., and Gross, L.: Arch. Int. Med. **54**:170, 1934.
6. de Vecchi, B.: Arch. Path. **12**:49, 1931.
7. White, P. D.: Am. J. Dis. Child. **32**:536, 1926.

occurs it is more likely to be associated with septic than with rheumatic infections. The process is primarily valvulitis followed by superficial necrobiosis, thrombosis and vegetation. Microscopic damage with fibroblastic proliferation and palisading may exist in the valve leaflet without any development of vegetative lesions.

The rash which suggested scarlet fever in the early stage of the disease may well have been an erythematous manifestation of the inception of rheumatic fever (Osler [8]).

The hyperleukocytosis (76,000), which led to the mistaken clinical diagnosis of myelogenous leukemia, may be attributed to the thrombosis of the coronary artery. The usual finding in coronary thrombosis is moderate leukocytosis, but occasional cases have been recorded in which the reaction was excessive. Hines [9] cited a case of coronary thrombosis in a Negress of 65 years in which the leukocytosis reached 140,000 with no evidence of leukemia at autopsy. In another case, reported by Levine,[10] the leukocyte count was 34,500.

Aneurysms of the coronary arteries are rare. Recorded instances in which the condition was of mycotic or of arteriosclerotic origin, as collected by Packard and Wechsler,[11] total 30. Occasionally congenital aneurysmal dilatations are found but are usually associated with an irregular distribution of the coronary vessels or other congenital anomaly of the heart (Harris [12]). The possibility of the aneurysms in the present case being of congenital origin suggests itself and cannot be denied or proved because of the inflammatory reaction which dominates the picture. However, because of the extensive acute exudative and destructive lesions in the walls of the vessels and because of the absence of other congenital defects in the heart, the aneurysms are considered of inflammatory origin.

SUMMARY

A case of acute rheumatic fever with myocarditis, auriculitis, pericarditis, microscopic endocarditis and aortitis in a young child is presented. Marked acute inflammatory lesions of proliferative, degenerative and necrotic character involved the main coronary arteries. Large aneurysmal dilatations in the right coronary artery and the descending branch of the left coronary artery are believed to have been the result of inflammation and manifested a very unusual development in the course of rheumatic fever. Thrombosis occurred in the right coronary aneurysm without causing infarction of the myocardium. The hyperleukocytosis is considered to have been a reaction to the thrombosis.

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8. Osler, W.: Principles and Practice of Medicine, ed. 12, New York, D. Appleton-Century Company, Inc., 1935.

9. Hines, D. C.: California & West. Med. **38**:372, 1933.

10. Levine, S. A.: Medicine **8**:245, 1929.

11. Packard, M., and Wechsler, H. F.: Arch. Int. Med. **43**:1, 1929.

12. Harris, P. H.: Am. J. Path. **13**:89, 1937.

Notes and News

Theobald Smith Prize.—This prize of $1,000, established by Eli Lilly & Company, has been awarded by the American Association for the Advancement of Science to Robley B. Evans, of the Massachusetts Institute of Technology, for his method of detecting radium poisoning.

University News, Promotions, Resignations, Appointments, Deaths, etc.—In the University of Chicago, Paul R. Steiner has been made assistant professor of pathology.

Jakob Erdheim, professor of pathologic anatomy in the University of Vienna, died suddenly on May 1 at the age of 65 years. Soon after graduating in medicine at the University of Vienna in 1913, he began work in pathologic anatomy under Anton Weichselbaum. In 1916 he became professor of pathologic anatomy and in 1924 director of the Institute of Pathologic Anatomy and Bacteriology of the Hospital of the City of Vienna. He made outstanding contributions to the knowledge of tumors of the hypophysial duct, of gestational changes in the hypophysis and of the parathyroids in malacic diseases of bones, and to the fundamental morphology of the bone in Paget's disease. His monographs "Rickets and Parathyroid," 1914, and "Vital Processes in Normal Cartilage and Its Proliferation in Akromegaly," 1931, are modern medical classics. Erdheim's demonstration course was one of the most stimulating experiences for American physicians doing postgraduate work in continental Europe.

Francis Harbitz, professor of pathologic anatomy and general pathology in the University of Oslo since 1900, having reached the age limit, has retired. The June number of *Norsk magasin for laegevidenskapen* is issued as *Festschrift* dedicated to him.

Announcement has been made of the resignation of George B. Magrath as professor of legal medicine in Harvard University Medical School, Boston.

The retirement has been announced of W. W. Ford as professor of bacteriology in the School of Hygiene and Public Health of the Johns Hopkins University.

Gaylord W. Anderson, Boston, has been appointed professor of preventive medicine and public health in the University of Minnesota.

Donald C. Balfour has been appointed director of the Mayo Foundation of the University of Minnesota in the place of Louis B. Wilson, who becomes director emeritus.

John E. Gordon, now field director of the International Health Division of the Rockefeller Foundation, has been appointed professor of preventive medicine and epidemiology in the Harvard University Medical School.

Robert M. Moore, assistant professor of pathology, University of Missouri School of Medicine, Columbia, has been appointed professor of pathology and director of clinical laboratory diagnosis in the University of Mississippi School of Medicine, effective September 1, it is reported.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Longitudinal Overgrowth of Long Bones with Special Reference to Fractures. J. D. Bisgard, Surg., Gynec. & Obst. **62**:823, 1936.

It has been observed that in young children a fractured extremity with as much as 2 or 3 inches (5 or 7 cm.) of shortening may in time become appreciably longer than its fellow. In an effort to learn the reason, experiments were made on young kids. It was found that lengthening in excess of normal occurs in growing bones after fracture, extending over a period of at least seven months. The gain in length takes place at the ends of the shafts and is due to increased activity in the processes responsible for normal growth. Overgrowth takes place without regard to the length of the bone or the necessity of equalizing the length of the extremities; in the absence of shortening, the fractured bone becomes longer than the normal one. Localized overgrowth is commonly confined to the fractured bone, and for this reason it is felt that the stimulus responsible for it is also local. In the absence of fracture, shortening alone causes no acceleration of growth. It must be conceded that the fracture and the reparative processes supply the stimulus. Experimental results suggest that the stimulus is merely incidental to the prolonged abnormal status of the part occasioned by the fracture and subsequent repair.

Warren C. Hunter.

Investigations on Respiratory Dust Disease in Operatives in the Cotton Industry. C. Prausnitz, Medical Research Council, Special Report Series, no. 212, London, His Majesty's Stationery Office, 1936.

The carding room dust is rich in particles under 2 microns in size and still richer in ultramicroscopic particles. Such particles can easily penetrate into the deeper air-passages. The lipoid fraction of the dust appears to be harmless to the lungs. The alcoholic fraction contains appreciable amounts of a substance resembling histamine and possibly identical with it. Similar substances have been found in cotton seed as well as in the dust of barley, esparto-grass, coir and wool. Whether this principle plays a pronounced rôle in causing the respiratory disease of cotton operatives is doubtful. The protein fraction has been shown by experiments on man and animals to be primarily toxic; it has a characteristic irritating effect on the deeper tissues and produces definite inflammatory lesions. In all probability the substance or group of substances contained in the protein fraction is responsible for the changes occurring in the lung after prolonged inhalation of the dust. Allergic supersensitiveness to the protein has been demonstrated to a greater or lesser degree in all the diseased cotton operatives examined; it is rare in healthy cotton operatives, in patients suffering from spasmodic asthma and in healthy persons unconnected with the cotton trade. Physiologic tests have shown definite impairment of respiratory function in a large proportion of cotton operatives suffering from the respiratory disease. Treatment of the disease by desensitization does not appear to provide satisfactory chances of improvement. Physical exercises directed toward improving the patient's respiration give benefit. The greatest importance attaches to removal of the patient from the injurious conditions of his work before severe secondary changes have developed in the lungs. The leading principle in dealing with this disease, as with all industrial maladies, must be prevention. The ideal requirement would be to keep the air of the mixing, blowing and carding rooms free from dust. Much has been accomplished in this direction, and yet the disease has not been eradicated. This appears to be due to the fact that even powerful, well directed ventilation fails to act on the

lightest and smallest particles of dust, and it is these which have the greatest power of penetrating into the deeper portions of the lung. So long as this technical problem remains unsolved, it will be necessary to provide adequate respirators and insist on their constant use during the dusty part of the work.

FROM THE AUTHOR'S SUMMARY.

CHANGES IN THE CAPILLARIES OF THE MYOCARDIUM IN EXPERIMENTAL EPINEPHRINE SCLEROSIS. B. D. IWANOWSKY, Virchows Arch. f. path. Anat. **297**:100, 1936.

In the normal myocardium of the rabbit the capillary endothelium in general lies in direct contact with the sarcolemma of the muscle fibers. Only here and there does one see an adventitial cell external to the endothelium as a representative of the mesenchyme, which may become activated. In young rabbits that received repeated intravenous injections of epinephrine, there were observed degenerative changes in the media of the arteries and of the precapillary arterioles and compensatory proliferation of the intima. In the capillaries there was thickening of the argentophil inner limiting membrane of the endothelium; this change is ascribed to a metamorphosis of the endothelial protoplasm. External to this membrane was a second one also impregnated with silver, not seen in the normal capillary; this is formed by the adventitial cell mesenchyme. The latter tissue also gives rise to fibrillated connective tissue that surrounds the capillaries.

O. T. SCHULTZ.

ORGAN CHANGES EXPERIMENTALLY INDUCED BY THYROXIN. H. HEINLEIN and I. DIECKHOFF, Virchows Arch. f. path. Anat. **297**:252, 1936.

Thyroxin, in dosages of 0.25 to 0.75 mg. per kilogram, was administered intravenously daily or every other day to twenty-five cats. The larger doses led to the death of the animals in a short time, with little or no detectable change in the organs. In experiments of longer duration the myocardium was severely damaged. The muscle fibers revealed cloudy swelling, fatty change, hyalinization, fragmentation and necrosis. The cellular infiltration present about the necrotic fibers is held to have been the result of reaction to the necrotic material. Electrocardiographic changes were observed. In the liver there were seen focal areas of capillary dilatation with endothelial desquamation and degenerative changes in the adjacent liver cells. Such alterations are held to be the precursors of those noted by Rössle in the human liver in exophthalmic goiter. In the kidneys there were degeneration and necrosis of tubular epithelium. No changes were observed in the thyroid gland or spleen.

O. T. SCHULTZ.

Pathologic Anatomy

THE RELATION OF AGE AND HYPERTENSION TO THE STRUCTURE OF THE SMALL ARTERIES AND ARTERIOLES IN SKELETAL MUSCLE. F. C. ANDRUS, Am. J. Path. **12**:635, 1936.

In specimens of pectoral muscle from 137 persons a marked variation in the degree of fibrosis of the medial of the vessels was observed in sections stained with the Mallory-Heidenhain stain. The degree of fibrosis was most marked in severe hypertension. There was a somewhat greater average amount of fibrous tissue in the walls of the arterioles in hypertensive than in nonhypertensive patients of the same age group. All persons with hypertension did not show marked fibrosis. No cases of marked fibrosis were seen in persons less than 29 years of age. In persons older than this it was encountered with increasing frequency. However, many persons of advanced age show only minimal degrees of fibrosis. The connective tissue fibers apparently arise from the reticulum and collagenous fibers in the media. The ratio of wall to lumen is determined by the contraction of the vessel, which can be estimated by the length and tortuosity of the internal elastic

lamina. Intimal disease was not found in the small arteries or arterioles of pectoral muscle at any age. It was not seen in any case of hypertension. One could not distinguish between hypertensive and control patients by examination of the small arteries and arterioles of pectoral muscle.

FROM THE AUTHOR'S SUMMARY.

MENINGITIC MYOCARDITIS. O. SAPHIR, Am. J. Path. **12**:677, 1936.

Observations on isolated meningococcic myocarditis not accompanied by meningococcic endocarditis are rare, as far as one can judge from the relevant literature. However, because of the fact that meningococcic myocarditis was found twice among ten cases of meningococcic meningitis, which were carefully studied for inflammatory changes in the heart muscle, the disease is apparently not so rare as one would expect from the literature. Two cases of meningococcic myocarditis are reported. In one, the patient showed signs of meningitis and died with marked cyanosis about fifty hours after the onset of symptoms. The second patient, who also had typical symptoms of meningitis, had apparently improved when cyanosis developed and death occurred unexpectedly. Histologically, meningococcic myocarditis is characterized by hemorrhagic exudation, early appearance of endothelial leukocytes, destruction of muscle fibers and presence of intracellular gram-negative diplococci. There are also foci of necrosis, which, however, are similar to those seen in the myocardium in subacute bacterial endocarditis.

FROM THE AUTHOR'S SUMMARY.

PRIMARY DEGENERATION OF THE CORPUS CALLOSUM (MARCHIAFAVA'S DISEASE). L. S. KING and M. C. MEEHAN, Arch. Neurol. & Psychiat. **36**:547, 1936.

The authors' patient, aged 56, was, as were the patients in all the other forty-two cases reported, addicted to the use of alcohol and of Italian extraction. For three years he exhibited mental changes (moroseness, irritability), which were followed by disturbances of speech, unsteadiness of the gait and paralysis of the extremities, which became spastic, with the reflexes exaggerated, but without pathologic signs. Necropsy revealed symmetrical degeneration of the corpus callosum (of the central lamina), anterior commissure, optic chiasm and subarcuate region of the temporal and occipital lobes. Microscopically, the degenerated areas exhibited demyelinization; the axons for the most part were retained, even in areas consisting of fat granule bodies, but were often swollen and varicose; other changes were absence of gliosis, regressive changes in the astrocytes, proliferation of the capillaries and absence of inflammatory phenomena. Markedly demyelinized areas exhibited no fully formed fat granule bodies but showed mainly microgliocytes and oligodendrocytes.

G. B. HASSIN.

CEREBRAL FRONTAL AGENESIS IN EPILEPSY. J. F. BATEMAN, Arch. Neurol. & Psychiat. **36**:578, 1936.

Of 178 brains of persons who had had convulsions, Bateman found gross anatomic changes in 174. Two brains were normal. Two showed anomalous blood vessels. In 146 brains from persons whose condition was diagnosed as idiopathic epilepsy the most frequent gross anatomic change was bilateral frontal atrophy, especially in brains from persons in whom the onset of the convulsions occurred before or during pubescence. Next in frequency was venous engorgement. Less common were, among others, unilateral cerebral agenesis, atrophy of the frontal, temporal or occipital lobes, dilatation of the cerebral ventricles and hyperplasia of the pacchionian bodies. The latter condition is not considered by Bateman among possible causes of epileptic convulsions or of the cortical atrophy, as such granulations commonly occur in persons without nervous or mental disorders and seem not to affect the parietal lobe, where they are especially numerous. The cortical atrophy in epileptic patients is, according to Bateman, a manifestation of cerebral cortical agenesis.

G. B. HASSIN.

THE PRIMARY LESION IN PHOSPHATE NEPHRITIS. J. B. DUGUID, J. Path. & Bact. **43**:321, 1936.

The development of chronic phosphate nephritis is discussed with special reference to the theory of the selective action of poisons on special segments of the renal tubules. The experimental evidence produced seems to be against this theory. When large doses of acid sodium phosphate are injected into rats, the whole of the first convoluted tubule appears to be more or less affected, and no evidence of selective action on any particular part of it is seen. There is, however, a tendency for the terminal segments of these tubules to become packed with solid material derived from fragmentation of the epithelium higher up, and it is suggested that the zonal calcification of chronic phosphate nephritis is a development of this kind rather than evidence of a selective action.

FROM THE AUTHOR'S SUMMARY.

THE NATURE OF THE PULMONARY ALVEOLAR LINING. M. E. GAZAYERLI, J. Path. & Bact. **43**:357, 1936.

The alveoli of the lung possess an epithelial lining which consists of flat nucleated cells. In addition, certain cuboid cells ("septal cells") are found, which have been demonstrated to have a phagocytic function and to stain specifically with del Rio Hortega's silver carbonate. These are part of the reticulo-endothelial system and give rise by mitosis to the free alveolar phagocytes.

FROM THE AUTHOR'S SUMMARY.

CHROMATOPHORES IN SYRINGOMYELIC CAVITIES. T. ALAJOUANINE and T. HORNET, Ann. d'anat. path. **12**:897, 1935.

In a case of syringomyelia microscopic sections of the spinal cord revealed great numbers of chromatophores containing melanin within the spinal cord about the syringomyelic cavities. There was evidence of active phagocytosis by these chromatophores. Apparently this is the only case in the literature in which melanin-containing chromatophores, normally present only in the pia mater, have been found in the interior of the nervous system. The authors believe that the melanin, a derivative from amino-acid, developed from products of the destruction of tissue in the course of the formation of the cavities.

PERRY J. MELNICK.

BENIGN SUBCUTANEOUS LIPOGRANULOMA DEVELOPING AFTER DIATHERMY, TRAUMATISM OR HYPODERMIC INJECTIONS. E. C. CRACIUN and J. FAGARASANO, Ann. d'anat. path. **12**:909, 1935.

Three cases of nonspecific granulomatous conditions of subcutaneous fat tissue are reported. Although trauma is the most frequent cause of this condition, only one of these cases followed trauma; the other two followed diathermy and hypodermic injection, respectively. The authors point out that the process does not always depend on an initial necrosis of fat but may consist of cellular reactivity from the beginning.

PERRY J. MELNICK.

HISTOLOGIC CHANGES IN THE DOG'S PANCREAS UNDER THE INFLUENCE OF SECRETIN AND OF EXTRACT OF DUODENAL MUCOSA. G. ALBOT and M. BOLGERT, Ann. d'anat. path. **12**:919, 1935.

Histologic examination of the pancreases of dogs given injections of secretin and other dogs given an extract of duodenal mucous membrane revealed the same characteristics of the secretion granules, etc., as are observed during digestion. The same conclusion was reached by observing the pancreatic secretion after

cannulating the duct. The action of these substances on the pancreas appears to be a normal physiologic one and is quite different from that of pilocarpine on the pancreas. The latter substance induces a state of marked hyperfunction.

PERRY J. MELNICK.

THE TESTES OF SEXUAL PERVERTS. R. RÖSSLE, Virchows Arch. f. path. Anat. **296**:69, 1935.

Gross and microscopic examination of the testes of 125 men legally castrated because of sexual misconduct revealed no deviation from normal. Legalized castration in such cases removes normal organs. But since sexual perversion has its basis in hypersexuality, the removal of a normal organ which mediates the sexual conduct is a warrantable procedure.

O. T. SCHULTZ.

INFECTIOUS REACTIVE RETICULO-ENDOTHELIOSIS AS A HYPERERGIC MANIFESTATION. A. SYMEONIDIS, Virchows Arch. f. path. Anat. **296**:497, 1935.

A general discussion of the reticulo-endothelioses, with their classification into storage, infectious, hyperplastic and dysplastic forms, is followed by a detailed report of a case interpreted as one of an infectious reactive reticulo-endotheliosis. The clinical picture was indefinite but was held to be that of an infection. There was moderate leukocytosis with relative monocytosis. The spleen and liver were enlarged. Histologic examination post mortem revealed minute perivascular granulomas of the spleen, liver, bone marrow and lymph nodes. The process was held to be a disease of the reticulo-endothelial system. The histologic appearances might warrant use of the term "pseudolymphogranulomatosis." The earliest lesion, seen in the spleen, was a minute nodule or area of fibrinoid degeneration of the reticulum fibrils. This form of degeneration, together with proliferation of the endothelium of small blood vessels and perivascular granulomatous proliferation of reticulo-endothelial cells, leads to the interpretation of the findings as a hyperergic reaction to infection, probably streptococcic in origin.

O. T. SCHULTZ.

INCIDENCE OF METOPISM IN NORTHERN NARONIA AND SILESIA. G. F. MATERNA, Virchows Arch. f. path. Anat. **296**:599, 1936.

Metopism (failure of closure of the frontal suture of the skull) was said by Welčker to occur in from 1 in 54 to 1 in 8 of all persons, the incidence being greatest in the European races. According to LeDóuble, the frequency varies from 1 to 8.2 per cent, with the highest incidence in the white races and 1 and 1.2 per cent in native Australians and in Negroes, respectively. Materna's study was based on 3,000 necropsies of persons over 2 years old during the years 1928 to 1935, inclusive. Metopism was observed in 61, or 2.03 per cent. Part of the material came from general hospitals and part from hospitals for patients with mental disorders. Of 1,819 necropsies in general hospitals, 1.81 per cent disclosed metopism; of 1,181 institutions for patients with mental disorders, metopism was observed in 2.37 per cent. Materna thinks this difference may have some significance.

O. T. SCHULTZ.

HEMORRHAGES INTO THE FLOOR OF THE FOURTH VENTRICLE IN INTOXICATIONS AND INFLAMMATIONS OF THE BRAIN. O. BERNER, Virchows Arch. f. path. Anat. **296**:636, 1936.

Berner had previously described the occurrence of multiple minute hemorrhages in the floor of the fourth ventricle following trauma to the head. Such hemorrhages were observed when the trauma and other evidences of injury to the brain were slight. They were usually situated just anterior to the striae acusticae and beside the median sulcus. They led him to state that the finer vessels of this

region are especially susceptible to trauma. Duret had claimed that such hemorrhages are due to rupture of small vessels caused by the mechanical shock of the injury. Berner claimed that they are the results of diapedesis. If the latter is true, similar lesions should be seen in nontraumatic conditions. He describes briefly the findings so far as hemorrhages into the floor of the fourth ventricle are concerned in five cases of acute methyl alcohol intoxication, one case of acute carbon monoxide poisoning and one case of acute poliomyelitis. The presence of hemorrhages in these conditions as well as after trauma leads Berner to maintain his position that the vessels of the floor of the fourth ventricle are more disposed to diapedesis than the vessels of the rest of the brain. O. T. SCHULTZ.

FORMATION OF PANCREATIC ISLETS FOLLOWING DUCT OBSTRUCTION DUE TO CARCINOMA OF THE HEAD OF THE PANCREAS. L. BURKHARDT, Virchows Arch. f. path. Anat. **296**:655, 1936.

In two cases of carcinoma of the head of the pancreas with obstruction of the duct, the acinar tissue had undergone atrophy. From the terminal intralobular ducts there had developed cellular structures which Burkhardt considers new-formed islets. Most of these islets were atypical in structure. The histologic picture was like what has been described as islet formation after experimental ligation of the pancreatic duct. Degenerative changes were common in the new-formed tissue, which may account for the fact that both patients had clinical diabetes in spite of the new-formed islet tissue. O. T. SCHULTZ.

CEREBRAL CHANGES IN DELAYED DEATH AFTER HANGING AND CAROTID LIGATION. G. DÖRING, Virchows Arch. f. path. Anat. **296**:666, 1936.

Many of the changes that occur in the brain have been ascribed by Spielmeyer and his followers to circulatory disturbances. In such cases the circulatory alteration has been of more or less prolonged duration. When cessation of the cerebral circulation leads immediately to death, few changes other than capillary hemorrhages are observed. Döring describes the early histologic changes in two cases. In one, death occurred ninety-six hours after an attempt at suicide by hanging; in the other, fifty-eight hours after ligation of the right common carotid artery. In each case there were multiple necroses of the cerebral cortex with no characteristic localization. The necrosis of the basal ganglions was of the coagulation type. The globus pallidus was severely damaged; in the death due to hanging this change was bilateral, and in the other death it was limited to the side of ligation. The ganglion cells revealed acute degenerative changes of varying degrees; the glia, only early regressive changes. O. T. SCHULTZ.

CONGENITAL MUSCULAR HYPERTROPHY OF AN UPPER EXTREMITY. H. VON BRÜCKE, Virchows Arch. f. path. Anat. **296**:681, 1936.

A male infant born one month prematurely presented no external maldevelopments except enlargement of the left upper extremity. The increase in size involved chiefly the forearm and hand. The circumference of the left arm was was 9.5 cm. as compared with 8.8 cm. for the right arm; the left forearm was 11.2 cm. in circumference, the right 8.6 cm.; the left hand measured 11 cm. in circumference, the right 7.7 cm. The two upper extremities were approximately equal in length; the bones were equal in length, but those of the left arm were somewhat thicker. The increase in the size of the hand appeared to be due largely to subcutaneous fat, but that of the forearm and arm, to muscular hypertrophy. The muscular strength on the enlarged left side was greater at birth and at a subsequent examination three months later. Von Brücke places the anomaly in the group termed "sector maldevelopments," in which the malformed portion is

usually of less functional value than normal. In the present instance, the greater muscular strength is indicative of an excessive formation of normally functioning tissue. The possible later development of evidences of neurofibromatosis is considered.

O. T. SCHULTZ.

Microbiology and Parasitology

THE VIRUS OF INFLUENZA. A. R. DOCHEZ, K. C. MILLS and Y. KNEELAND JR., J. Exper. Med. **63**:581, 1936.

Evidence is presented indicating that a filtrable virus is present in the nasopharyngeal secretions of persons suffering from influenza. An attempt to transfer influenza from one person to another by means of filtered nasopharyngeal washings resulted in the production of a common cold. From filtered nasopharyngeal washings of patients with influenza a filtrable agent was cultivated in a tissue medium. Inoculation of the cultivated virus into human volunteers produced for the most part severe common colds with a tendency toward pronounced constitutional reactions. In one person an infection clinically resembling influenza was produced. The more closely the source of the virus approached the type of epidemic influenza the more likely the virus was to provoke constitutional symptoms. The presence of certain pathogenic bacteria in the upper respiratory tract was not observed to modify the course or character of the experimental infection in the inoculated volunteers.

FROM THE AUTHORS' SUMMARY.

EFFECT OF MUCIN ON BACTERIAL INFECTIONS. W. J. NUNGESTER, L. F. JOURDONAIS and A. A. WOLF, J. Infect. Dis. **59**:11, 1936.

When sterilized gastric mucin is used instead of saline solution as a menstruum for suspending various bacteria, the host is placed at a marked disadvantage and may succumb to what would otherwise be a sublethal infection. This effect has been noted when injections were made intraperitoneally, subcutaneously or intratracheally.

The mechanism of the action is not yet understood. Mucin does not interfere with phagocytosis but inhibits the bactericidal properties of phagocytic cells. It enables bacteria to survive in the body of the host for longer periods without diminution in numbers, or it may allow the organisms to increase in numbers and so bring about the death of the animal. The viscosity and cohesiveness of the mucin appear to be important indexes to its effectiveness in promoting bacterial infections.

FROM THE AUTHORS' SUMMARY.

THE RECOVERY OF VIRULENT TUBERCLE BACILLI FROM THE TISSUES OF SWINE INTENDED FOR FOOD. W. H. FELDMAN, J. Infect. Dis. **59**:43, 1936.

Attempts were made to isolate and type tubercle bacilli that might be present in dressed carcasses of swine which had been retained following postmortem inspection for tuberculosis. Material was obtained only from those carcasses in which the lesions of tuberculosis were slight or localized and which were "passed for food" after the diseased tissues had been removed. The left precrural lymph node from each of 88 carcasses and the right internal iliac lymph node from each of 13 carcasses were obtained and cultures prepared. Of the total of 101 specimens, tubercle bacilli were obtained from 4. Studies of pathogenicity revealed all of the strains to be Mycobacterium tuberculosis-avium.

This study should emphasize anew the importance of the problem of avian tuberculosis and should indicate that virulent tubercle bacilli may still be present in the carcasses of hogs even though all visible lesions of tuberculosis have been removed.

FROM THE AUTHOR'S SUMMARY.

THE CELLULAR REACTIONS DURING PRIMARY INFECTIONS AND SUPERINFECTIONS OF PLASMODIUM BRASILIANUM IN PANAMANIAN MONKEYS. W. H. TALIAFERRO and P. R. CANNON, J. Infect. Dis. **59**:72, 1936.

Defense against Plasmodium brasilianum in various species of Panamanian monkeys involves chiefly (*a*) suppression of the infection and (*b*) replacement of the erythrocytes lost by schizogenesis of the parasites and by erythrophagia and, to a less extent, lymphocytes lost by lymphorrhexis.

Suppression of the infection is purely parasiticidal except for an occasional transitory inhibition of reproduction at the crisis. It resolves itself into phagocytosis of parasites, both extracorpuscular (merzoites free in the plasma) and intracorpuscular (all stages). Phagocytosis is accomplished by macrophages, primarily of the spleen, to a less extent of the liver and to a still less extent of the bone marrow. Macrophages of other organs are unimportant.

The development of the primary infection can be correlated step by step with the concentration and phagocytosis of the parasites as follows:

(*a*) During the acute rise, the parasites are concentrated in the spleen and to a less extent in the liver and bone marrow, where they are sluggishly phagocytosed. They are also concentrated in other organs to a degree comparable to that of their concentration in the liver and marrow but are not phagocytosed.

(*b*) At the crisis, the parasites are *regionally* concentrated in the spleen. They are held in the Billroth cords and do not pass into the venous sinuses. This suggests that they either are agglutinated or adhere to the macrophages. Then, within a day or two (sometimes more), they are aggressively ingested by the macrophages. They are likewise more actively ingested by the macrophages of the liver and bone marrow, but the structure of these two organs is not conducive to ascertaining whether here they are also regionally concentrated. The macrophages at this time are engorged with all forms of the parasites in all stages of disintegration. The organized red cells and parasites rapidly disappear, but the pigment remains for some time.

(*c*) Once critical phagocytosis is initiated, the infection is usually subdued. At this time the macrophages are filled with pigment. The pigment disappears in about three months, provided it does not reaccumulate as the result of relapses.

(*d*) Following splenectomy there is a compensatory increase in phagocytosis in the liver and bone marrow, and occasionally, an increase in pigmentation in the lungs.

After superinfection with large numbers of parasites, the active concentration and phagocytosis of the parasites begins within one hour instead of from one to several weeks. In other words, immunity takes time to develop, but once acquired, responds immediately.

Acquired immunity, whether as a manifestation of recovery from the initial infection or as a response to superinfection, depends primarily on (*a*) the greatly increased rate of phagocytosis by the individual differentiated macrophages, chiefly of the spleen, to a less extent of the liver and still less of the bone marrow, and (*b*) an increased number of macrophages in the spleen and bone marrow. The longer and more intense the malarial infection the more noticeable is the increase in the number of macrophages.

Concomitantly with the macrophage activity, if the infection is not checked, lymphoid hyperplasia occurs in the spleen. With an especially long and severe infection, it may even involve the liver and bone marrow. This acts as a mesenchymal reserve which replenishes and augments the supply of macrophages and lymphocytes. The transformation of lymphocytes into monocytes and macrophages can be particularly well seen in the spleen.

Replacement of erythrocytes is restricted to an enhanced erythropoiesis of the bone marrow. No ectopic erythropoiesis has been found.

FROM THE AUTHORS' SUMMARY.

EPIDEMIOLOGICAL ASPECTS OF SPONTANEOUSLY ACQUIRED VACCINIA IN THE RABBIT. L. PEARCE, P. D. ROSAHN and C. K. HU, J. Path. & Bact. **43**:299, 1936.

The development of generalized clinical vaccinia in unvaccinated rabbits exposed to rabbits vaccinated with dermovaccine (culture) is reported. The condition was similar to that observed in the vaccinated animals and had as its most conspicuous clinical manifestation a cutaneous papular eruption. The results of two experiments on large numbers of animals, carried out within three months of each other, were markedly different. In the first experiment the spontaneously acquired disease was comparatively severe, while in the second no case was observed. Similar differences in severity were observed in the vaccinated rabbits of both experiments, while in an experiment carried out midway between the two the infection of vaccinated animals was of a moderate grade. The progressive decline in severity is interpreted on the basis of variations in the reaction of the hosts. The epidemiological significance of such variations is pointed out.

FROM THE AUTHORS' SUMMARY.

PATHOLOGIC CHANGES DUE TO THE VIRUS OF LYMPHOCYTIC CHORIOMENINGITIS. G. M. FINDLAY and R. O. STERN, J. Path. & Bact. **43**:327, 1936.

The pathologic changes produced in susceptible animals by four strains of the virus of lymphocytic choriomeningitis isolated in this country from men and mice are described and found to be identical with those due to an American strain of the virus. Susceptible species are the mouse, rat, guinea-pig, monkey (Macacus mulattus) and crab-eating macacque (Macacus irus). Insusceptible species are the dog, pig, ferret, rabbit, field vole, bank vole, canary, hen and parakeet. The lesions in the central nervous system consist of meningitis and infiltration of the choroid plexus; infiltration with round cells may be found in the viscera, more especially the liver, adrenal glands and kidneys, together with bronchopneumonia. The acidophilic intranuclear inclusions very occasionally found in the cells of the choroid plexus of mice appear to be caused by the mouse salivary gland virus.

FROM THE AUTHORS' SUMMARY.

PASSAGE OF BACTERIOPHAGE FROM THE BLOOD TO THE SPINAL FLUID. M. KASAHARA and S. I. UYESHIMA, Zentralbl. f. Bakt. (Abt. 1) **136**:143, 1936.

The permeability of the blood-brain barrier of the rabbit was tested by the intravenous injection of bacteriophage to the typhoid bacillus and the simultaneous determination at varying intervals of the bacteriophage concentration in the blood and spinal fluid. The authors found that this bacteriophage invariably passed into the spinal fluid within two hours in normal rabbits, that it passed through more readily in young rabbits than in older ones and that preexisting meningitis produced by subdural injection of aleuronat increased the permeability of the blood-brain barrier.

PAUL R. CANNON.

THE BENEFICIAL ACTION OF GLYCERIN ON BACTERIUM DYSENTERIAE IN DYSENTERIC STOOLS. J. P. WU and R. H. P. SIA, Chinese M. J., supp., 1936, p. 179.

The viability of Bacillus typhosus in stools has been previously shown to increase markedly on the addition of glycerin. This finding has led to application of the same method to the handling of dysenteric stools intended for bacteriologic examination. A comparative study has been made of the isolation of Bacterium dysenteriae in dysenteric stools kept for varying periods of time with and without the addition of glycerin. Specimens of stools were collected as soon as they were passed. Each was immediately divided into two portions; to one portion 30 per cent glycerin in a 0.6 per cent saline solution was added in the proportion of not less than 2 parts of glycerin-saline solution to 1 part of feces, and to the other portion, which was to serve as a control, nothing was added. Both specimens were kept at room temperature, with only three exceptions, and were cultured at

regular intervals. The results of the study of fifteen stools are reported. In every case a positive culture was obtainable at a much later date from the portion in which glycerin-saline solution was added than from the control. The average prolongation of the period in which positive cultures were obtainable as a result of the addition of glycerin-saline solution was seven days (one day being the shortest and twelve days being the longest period). In two cases different concentrations of glycerin were also tried. The results indicated that 30 per cent was equally efficient, if not more so, than 20 and 50 per cent glycerin. The addition of 30 per cent glycerin in a 0.6 per cent saline solution to dysenteric stools is therefore recommended to increase the chance of obtaining a positive culture of Bact. dysenteriae, especially when examination is unavoidably delayed.

FROM THE AUTHORS' SUMMARY.

Immunology

THE MECHANISM OF IMMUNITY IN TUBERCULOSIS. M. B. LURIE, J. Exper. Med. **63**:923, 1936.

There is an extracellular factor which inhibits the growth of tubercle bacilli in immunized rabbits. Extracellular factors localize carbon particles, trypan blue and tubercle bacilli at the site of introduction to a greater extent in the immunized than in the normal animal. This greater fixation is brought about by an increase in the density and extent of the fibrin barrier formed about the focus of infection in the immunized animal. The more pronounced in vivo agglutination of tubercle bacilli and carbon particles in the vaccinated or tuberculous rabbit also tends to immobilize them in the tissues. The agents inhibiting growth of the injected bacilli and localizing them are effective on reinfection in the fixation of small doses at the portal of entry. With large doses, on reinfection, the increased lymph flow resulting from the intensified inflammation in the immunized animal brings about a more rapid dissemination of the bacilli to the draining lymph nodes than in the normal animal. The most significant factor in immunity is the increased capacity of the rapidly mobilized mononuclear phagocytes to destroy tubercle bacilli. The impotent polymorphonuclear leukocytes quickly disappear from the site of reinfection. The invading bacilli that reach the draining lymph nodes of the immunized animal are retarded in multiplication or destroyed by these phagocytes. Vaccination of rabbits with BCG brings into play the factors tending to immobilize the bacilli of reinfection, inhibit their growth and destroy them, with significant immunity resulting. A virulent primary infection affords greater immunity than an infection of low virulence, and the reactions of the host are expressed by a quantitative increase in those immunity factors which operate in a vaccinated animal.

FROM THE AUTHOR'S SUMMARY.

NATURAL IMMUNITY TO PNEUMOCOCCUS TYPE III. J. F. ENDERS and M. F. SHAFFER, J. Exper. Med. **64**:7, 1936.

A correlation appears to exist between the failure of certain strains of type III pneumococci to grow at 41 C. and their lack of virulence for rabbits. It is likely that the capacity to grow at 41 C.—an attribute constantly but not exclusively associated with strains of type III pneumococci virulent for rabbits—is a prerequisite but not the sole factor in determining their virulence in these animals.

FROM THE AUTHORS' SUMMARY.

CHEMO-IMMUNOLOGIC STUDIES ON CONJUGATED CARBOHYDRATE-PROTEINS. W. F. GOEBEL, J. Exper. Med. **64**:29, 1936.

Artificial carbohydrate-protein antigens containing the azobenzylglycosides of dextrose and glycuronic acid give rise in rabbits to antibodies which are distinct

and immunologically specific. The artificial antigen containing glycuronic acid reacts in high dilutions in antipneumococcic horse serums types II, III and VIII. The chemical basis for this serologic activity is discussed.

FROM THE AUTHOR'S SUMMARY.

IMMUNIZATION EXPERIMENTS WITH THE VIRUS OF SWINE INFLUENZA. R. E. SHOPE, J. Exper. Med. **64**:47, 1936.

Virus obtained from the lungs of ferrets or mice infected with swine influenza when administered intramuscularly or subcutaneously immunizes swine to swine influenza. Ferrets which have received subcutaneous injections of the swine virus from the lungs of infected ferrets are immune to intranasal infection with this virus. Similar injections of the virus from the lungs of infected mice or swine do not immunize ferrets. Mice can be immunized to intranasal infection with the swine virus by subcutaneous injection of the virus from the lungs of infected mice but not by similar injection of the virus from the lungs of infected ferrets or swine. Repeated injections induce greater immunity than a single one. Intraperitoneal injection of the swine virus from the lungs of infected mice, ferrets and swine into mice and ferrets immunizes them to intranasal infection, and it appears to make little difference in each case whether the virus is from infected mice, ferrets or swine. Field experiments in which swine influenza followed intramuscular injections of the virus are cited as demonstrating the hazard in this manner of immunization in a densely crowded susceptible population.

FROM THE AUTHOR'S SUMMARY.

THE FATE OF A STRAIN OF VIRULENT HEMOLYTIC STREPTOCOCCI INJECTED INTO THE SKIN OF NORMAL AND IMMUNIZED RABBITS. D. M. ANGEVINE, J. Exper. Med. **64**:131, 1936.

Virulent streptococci injected into the skin of normal animals multiply actively, resist phagocytosis, invade the tissues widely, enter adjacent and distant lymph nodes and in some instances are distributed by the blood stream to internal organs. After immunization associated with some sensitization, virulent streptococci are more readily ingested by phagocytes, remain sharply localized, are rapidly destroyed, fail to pass the nearest lymph nodes and do not enter the blood stream.

FROM THE AUTHOR'S SUMMARY.

THE IMMUNOLOGIC SPECIFICITY OF STAPHYLOCOCCI. L. A. JULIANELLE, J. Exper. Med. **64**:149, 1936.

Skin reactions to the type-specific carbohydrates A and B of Staphylococcus indicate that cutaneous sensitivity to type B is either extremely rare or nonexistent. Skin reactions to type A occur in about 12 per cent of normal infants and children and in from 65 to 70 per cent of normal adults. Skin reactions to type A occur regularly in patients with staphylococcic infection. Such reactivity is not associated with demonstrable serum precipitins, for (*a*) normal persons who were cutaneously reactive were found not to possess them and (*b*) not all patients with verified infection acquired them. The patients elaborating type-specific precipitins, who numbered less than 30 per cent in this study, were those with severe, prolonged or generalized staphylococcic infection. Specific carbohydrates were not found in the urine of patients during staphylococcic infection.

FROM THE AUTHOR'S SUMMARY.

VITAMIN C AND ANAPHYLACTIC SHOCK IN GUINEA-PIGS. B. SOLOMONICA, J. Immunol. **31**:209, 1936.

Daily injections of vitamin C prior to an intraperitoneal sensitizing injection of 1 cc. of a 1:100 dilution of horse serum and during the three week interval

until the intravenous test injection of 0.5 cc. of a 1:10 dilution of horse serum protected guinea-pigs against anaphylactic shock. The protection was less effective if the vitamin was administered only during the period of sensitization, but was evident even when the vitamin was injected intravenously simultaneously with the shocking dose.

I. DAVIDSOHN.

SERUM SICKNESS IN RABBITS. L. JONES and M. S. FLEISHER, J. Immunol. **31**:215, 1936.

Treatment of horse serum or pseudoglobulin with different lipid solvents removed in some cases more than 90 per cent of the lipids, and in most cases the removal is assumed to have been greater than 66 per cent. From comparison with controls and from quantitative considerations Jones and Fleisher conclude that serum lipids play no part in causing serum sickness in rabbits, although the decrease of incidence of serum sickness was considerable. The decrease was not due either to a denaturing effect on the serum proteins, as evidenced by a study of the antigenic properties of the treated serums, or to a variation in the reactive capacity of different lots of animals.

I. DAVIDSOHN.

AVAILABILITY OF SPECIFIC PNEUMOCOCCIC ANTIBODY AFTER INTRAVENOUS, INTRAMUSCULAR AND SUBCUTANEOUS INJECTIONS. R. C. TILGHMAN and M. FINLAND, J. Immunol. **31**:239, 1936.

The intravenous route was shown to be the one of choice for the administration of pneumococcic antiserum because in a comparative study of twenty-one adult patients suffering from diseases other than pneumonia the highest titer of circulating antibody was attained most rapidly and maintained at a higher level when the serum was given intravenously. Following intramuscular or subcutaneous injections, the concentration of antibody was as a rule only about 1 per cent of the concentration observed after intravenous administration. In the presence of a positive cutaneous and negative conjunctival reaction to the serum, an initial injection of the serum intramuscularly reduced the incidence of untoward reactions to subsequent intravenous injections.

I. DAVIDSOHN.

LOCALIZATION OF ANTIBODIES AND COLLOIDAL DYE IN INFLAMMATORY AREAS. J. P. FOX, J. Immunol. **31**:293, 1936.

The localization of intravenously injected trypan blue in inflammatory lesions, produced by intracutaneous injection of bacterial vaccines, calcium chloride or turpentine, was more rapid and more marked in the youngest lesions. The ability to localize the injected dye persisted to a lesser extent in inflammatory lesions produced by the stronger irritants. The localization of hemolytic and agglutinating antibodies, though less conclusively established, was presumed to occur. Its extent was determined by the age and severity of the inflammatory lesions about as in the case of the dye.

I. DAVIDSOHN.

THE BACTERICIDAL ACTION OF SERUM AGAINST MENINGOCOCCUS, GONOCOCCUS AND MICROCOCCUS CATARRHALIS. J. GORDON and L. HOYLE, J. Path. & Bact. **43**:537, 1936.

It is shown that the bactericidal action of serum toward meningococci, gonococci and Micrococcus catarrhalis is of the same nature as that previously demonstrated in the case of certain gram-negative bacilli. Factors inactivating complement, namely, heat, ammonia and congo red, also prohibit the bactericidal effect of serum, and, as was found with other bacteria, a heat-stable factor is necessary for the bactericidal action of serum against these organisms. The bactericidal action of the serum is therefore the result of the action of complement and heat-stable factor.

FROM THE AUTHORS' SUMMARY.

THE GENERAL BACTERICIDAL ACTION OF NORMAL SERUM. J. GORDON and L. HOYLE, J. Path. & Bact. **43**:545, 1936.

Adsorption of normal serum by dead bacteria in not too large amounts results in general weakening of the bactericidal power. All organisms are, however, killed by such an adsorbed serum, though more slowly than by normal serum. A specific effect is also shown, namely, that if an organism is tested against a series of adsorbed serums, it is killed most slowly by the serum which has been adsorbed by the same organism. It is argued that this specific effect is best accounted for as due to the introduction into the serum, during the adsorption, of a specific growth-promoting factor derived from the adsorbing organism itself and included in the products of its autolysis. This growth-promoting factor acts in opposition to that part of the bactericidal system which has escaped adsorption. If this explanation is valid, any adsorbed serum should also show maximum weakening of bactericidal power toward the adsorbing organism, provided that the serum is tested by organisms of equal sensitiveness. This is borne out by the results discussed. Evidence is brought forward against the existence of specific immune bodies in serum.

FROM THE AUTHORS' SUMMARY.

EFFECT OF PARTIAL IMMUNITY ON THE REACTION OF WHITE MICE TO THE VIRUS OF ENCEPHALITIS B (ST. LOUIS). C. ARMSTRONG and R. D. LILLIE, Pub. Health Rep. **51**:1069, 1936.

Normal mice when intracerebrally inoculated with the virus of encephalitis B usually presented a clinicopathologic picture pointing predominantly to localization of the virus in the brain. Partially immune mice when intracerebrally reinoculated with a proper dose of virus after a suitable interval tended to have symptoms and lesions pointing predominantly toward localization in the cord. There is destructive inflammation of the gray substance. Armstrong and Lillie failed to produce the predominantly myelitic symptoms when the second inoculation was made by the intranasal route. In some mice the paralyses were permanent; in others there occurred variable degrees of recovery. These observations are probably best explained by assuming that in mice the cells of the spinal cord are relatively more susceptible to the virus than are the cells of the brain and therefore require a higher degree of immunity to afford protection when once the virus has reached the cord. The possibility of a difference in response by the cells of the brain and spinal cord to active immunization cannot, however, be ruled out.

FROM THE AUTHORS' SUMMARY.

THE BLOOD CHARACTERISTICS M AND N. R. MARTINET, Arch. internat. de méd. expér. **11**:573, 1936.

Reactions occurred in seven patients after repeated blood transfusions. In each instance the blood of the donor contained the N characteristic and that of the recipient the M characteristic, or vice versa. The reactions consisted of mild shock with hemolytic icterus; they showed individual differences and even varied in the same patient. They were explained on the basis of anti-N or anti-M hemolysin unassociated with agglutinin. A caution is given to avoid repeated blood transfusions in the presence of such "crossed" bloods.

FREDERICK STENN.

PRODUCTION AND ABSORPTION OF ANTI-M AND ANTI-N IMMUNE SERUMS. S. OLBRICH, Ztschr. f. Immunitätsforsch. u. exper. Therap. **88**:63, 1936.

Forty-five rabbits inoculated intravenously from six to eight times at intervals of from three to four days with from 5 to 8 cc. of from 10 to 20 per cent suspensions of human red blood cells MO, with use of blood of the same person for each series of from six to eight rabbits, yielded twelve satisfactory immune serums—a percentage of 26.7. Twenty-five rabbits similarly treated with NO red corpuscles gave nine, or 36 per cent, satisfactory immune serums. The response

was dependent on the blood injected and on the individual properties of the animals. The titers were determined on slides by adding a drop of a 2 to 3 per cent suspension of red blood cells M, N or MN to each of a series of drops of increasing dilutions of inactivated serum. The results were read after from ten to twenty minutes. The absorption of the species-specific factors and the quality of the absorbed serums depended on the titer of the unabsorbed serum, on the readiness with which the species-specific antibodies were removed, on the absorbing quality and quantity of the red blood cells necessary for the absorption and on the resistance of the group-specific factors to nonspecific absorption. By varying (*a*) the dilutions of the serums during the absorption, (*b*) the quantity of absorbing red blood cells and (*c*) the number of absorptions it was determined that the final result varied in different serums. The lowest titer for the unabsorbed serum had to be 1:1,600 to yield a satisfactory absorbed serum. Olbrich recommends absorbing serum in dilutions of 1:5, 1:10 and 1:20 three times with one half, one third or one fourth of the volume of corresponding red blood cells and titrating after each absorption. An evaluation of the results of the absorptions permitted the selection of the optimal conditions for each immune serum. It was established that the titer of the absorbed serum had to be at least five dilutions higher for the homologous blood group than for the heterologous to be satisfactory, which means that its titer had to be at least 1:32. The serums were graded as group I, II or III according to the titers for the homologous and heterologous blood cells after the absorption. Only groups I and II were recommended for medicolegal purposes. Only fresh red blood cells are to be used for absorption. The length of absorption varied from one-half hour to twenty-four hours without apparent influence on the result. The effect of temperature did not differ in the range from 0 to 37 C.

I. Davidsohn.

Specificness of Gonadotropic Hormones and of Antiserums Against Them. R. Brandt and H. Goldhammer, Ztschr. f. Immunitätsforsch. u. exper. Therap. **88**:79, 1936.

Injections of the urine of pregnant women into rabbits resulted in the formation of two different and independent antibodies: (*a*) a complement-fixing antibody of short duration that reacted only with colloids of human urine and (*b*) an antihormone which inhibited the action of the gonadotropic substance. The antihormone was species specific; it inhibited the gonadotropic action of the serum of pregnant women and of extracts of human placenta and hypophysis but not that of the urine of mares. It was not dependent on the ability of the rabbits to produce the hormone, for it developed in response to injection of the hormone into rabbits that either had been castrated or were below the age and size necessary for the production of the hormone. The antibody character of the substances studied was further evidenced by the fact that they did not develop after injections of hormone from the same species. Brandt and Goldhammer expect that the study of antihormones will find a therapeutic and diagnostic application.

I. Davidsohn.

Production of Antibodies in Lymph Nodes. J. Murakami, Ztschr. f. Immunitätsforsch. u. exper. Therap. **88**:182, 1936.

The report deals with a comparative study in rabbits of complement and of actively produced and passively introduced antibodies in the lymph before the lymph enters a popliteal lymph node and after it leaves the node and in the blood serum. The complement was found to be highest in the blood serum and lowest in the outflowing lymph. The level of the antibodies in the lymph was dependent entirely on their quantity in the blood and was parallel to this quantity. An exception was present in the case of an inoculation of a typhoid vaccine into a toe, when the lymph flowing from the regional nodes had higher titers of antibodies than the blood, but only for a limited period, after which the titers returned

to the normal relation, which was found to be that of a slightly lower titer of antibodies in the outflowing lymph than in the blood. This finding indicates that the formation of antibodies takes place in the lymph node. The titer of the antibodies in the lymph entering the regional lymph nodes of an infected region is dependent on the level of the antibodies in the blood.

I. DAVIDSOHN.

THE THERMOSTABLE BACTERIOLYSINS. A. PETTERSSON, Ztschr. f. Immunitätsforsch. u. exper. Therap. **88**:210, 1936.

Normal blood serum contains, in addition to the specific bacteriolysins which require complement for their action, two other much more heat-resistant bacteriolysins: (*a*) a lysin acting on gram-positive bacteria (Bacillus mycoides, Bacillus subtilis, Bacillus anthracis, the Bacillus of Welch and Frankel and others), called by Pettersson betalysin, and (*b*) a lysin acting on the members of the colon-typhoid group. In addition, polymorphonuclear leukocytes contain a substance bacteriocidal for gram-positive and gram-negative bacteria. The betalysin was found to act at 0 C. almost as well, though somewhat more slowly as at incubation temperatures, and, furthermore, it was able to lyse larger numbers of bacteria at the lower temperature. It did not as a whole become absorbed by the bacteria, but one of its components, the so-called activating substance, became absorbed, while the second component, the activated substance, remained intact. The leukocytic bacteriolysins are not identical with the betalysin but have many features in common with the thermostable lysin directed against gram-negative bacteria.

I. DAVIDSOHN.

SPECIES-SPECIFIC ERYTHROCYTIC ANTIGEN IN HUMAN SERUM AND GROUP-SPECIFIC HEMAGGLUTININS IN THE CORRESPONDING PRECIPITATING ANTISERUMS. M. EISLER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **88**:240, 1936.

Human serums contain two species-specific antigens: (*a*) precipitinogen and (*b*) the species-specific antigen of the erythrocytes that is generally accepted to be identical with O agglutinogen. In addition, some serums contain either A or B antigens. In response to inoculations with human serum, rabbits showed the corresponding agglutinins. The quality of the response depended on the presence or absence in the animals of preformed agglutinins. The anti-O agglutinin that reacts with human bloods of all types appeared in the absence of normal anti-A and anti-B agglutinins in the rabbits; if the latter were present normally, they rose markedly after injection of proper human serums, and the titer of the species-specific anti-O agglutinin was low. The results were confirmed by separation of the absorbed agglutinins from the erythrocytes with heat according to the procedure of Landsteiner. Rabbits that were immunized with the serums of beef and sheep responded with anti-A agglutinin. The serums of these animals are known to contain the A substance. Rabbits treated with the serums of the hog, chicken and cat and with bacteria did not agglutinate human red cells.

I. DAVIDSOHN.

Tumors

STUDIES IN CARCINOGENESIS. M. J. SHEAR, Am. J. Cancer **28**:334, 1936.

A number of hydrocarbons have been tested for carcinogenic activity. In pure strain mice, approximately 10 mg. of crystalline hydrocarbon per mouse was introduced subcutaneously. 8,9-dimethylene-1,2-benzanthracene, a structural isomer of cholanthrene, was found to be carcinogenic but much more slowly acting than cholanthrene. 7-methyl-8,9-dimethylene-1,2-benzanthracene, a structural isomer of methylcholanthrene, was inactive. Two other hydrocarbons having the same empirical formula as methylcholanthrene were found to be inactive. 16,20-dimethylcholanthrene produced tumors at about the same rate as 1,2,5,6-dibenzanthracene. 1,9-methylene-1,2,5,6-dibenzanthracene was also found to be carcinogenic; it produced tumors more slowly, however, than 1,2,5,6-dibenzanthracene. 15,16-benzdehydrocholanthrene, tested on fourteen mice, produced only one tumor in ten months.

FROM THE AUTHOR'S SUMMARY.

FURTHER QUANTITATIVE METHODS FOR THE STUDY OF TRANSPLANTABLE TUMORS. R. SCHREK, Am. J. Cancer **28**:345, 364, 372 and 389, 1936.

Using careful measurements of tumor size as ordinates and time as abscissas, Schrek obtained curves for the rates of growth of R39 rat sarcoma and the Brown-Pearce rabbit carcinoma. With the former, four types of curve were observed: linear, curvilinear, regressive and irregular. With the Brown-Pearce tumor, only two types were seen, curvilinear nonprogressive and regressive. The linear curves express essentially the growth constant of the tumor without modifying factors. The curvilinear tumors show the effect of presumable acquired immunity, and their growth may be expressed mathematically as an exponential equation, involving the introduction of an inhibition constant. When this becomes zero, the curve becomes rectilinear. Analyzing his results, Schrek concludes: Tumor growth is an orderly physiologic process which may be divided into three components—a latent phase, a growth impulse and an inhibitory factor. The average growth constant is physiologic for the tumor strain.

Similar work with inoculums of various sizes showed the following results: The smaller the inoculum the less is the percentage of successful takes, the greater is the latent period and, to a slight degree, the smaller is the growth constant. There was no appreciable effect on the percentage of regressions or on the inhibition constant.

With rat tumors 256A, 256B, R39 and the Flexner-Jobling tumor, fortuitous variations in general were small. With the last, it was possible to compare the present rate of growth with measurements made fifteen years ago; the rate is still approximately the same. With R39, there was marked variation in percentage of takes, average latent period and percentage of regressions, indicating the presence of some complicating factor, presumably the immune response of the host. With none of the tumors was anything observed to indicate seasonal variation or rhythmic variation of the rate of growth. With Walker carcinoma 256, permanent variations in the rate of growth were observed in the form of a sudden increase, along with an increase in the percentage of takes and a diminution in the period of latency. Schrek believes that this change is similar to that by which normal tissues and benign tumors become malignant. As regards fortuitous variations, the factors responsible are errors in measurement, variations in technic, variations in the animal used, seasonal variations and variations in the tumor itself. In his own work, variations of this sort could be attributed entirely to errors of measurement and to fluctuations in technic and in animals.

Cell suspensions of Walker tumor 256, sarcoma R39 and rat testis were prepared by grinding the tissue in a Latapie apparatus and filtering through 80 mesh Monel wire cloth; these suspensions were diluted twenty-five times with Tyrode solution, p_H 7.6, containing eosin (1:2,000). Unstained cells were then counted and evaluated by means of a hemocytometer. Moccasin venom, 1,200, had an immediately deleterious effect on Walker tumor cells, as determined by viable cell counts; ricin, 1:200, had no immediately injurious effect; exposure of Walker tumor cells to a temperature of 43 C caused slow diminution of viable cells.

H. E. EGGERS.

CARCINOMA OF THE BREAST IN HOMOLOGOUS TWINS. S. A. MUNFORD and H. LINDER, Am. J. Cancer **28**:393, 1936.

Carcinoma of the breast occurring simultaneously in the same position in homologous twin sisters is reported.

SYNOVIAL SARCOMA. L. C. KNOX, Am. J. Cancer **28**:461, 1936.

Synovial sarcoma is a rare form of malignant tumor of an extremity. Abstracts of reports of nineteen such cases in the literature are included here, and three new cases are reported. Malignant tumors of the joints, bursae and tendons may usually be separated into one of two groups, fibrosarcoma or synovial sarcoma.

The latter are recognized microscopically by the epithelial-like cells lining tubules, pseudoglands or clefts, outside of which exists a dense feltwork of connective tissue. Synovial sarcoma is apparently resistant to radiation, nor have any permanent cures by amputation been recorded.

FROM THE AUTHOR'S SUMMARY.

INCREASED PHOSPHATASE ACTIVITY OF BONE AT THE SITES OF OSTEOPLASTIC METASTASES SECONDARY TO CARCINOMA OF THE PROSTATE GLAND. E. B. GUTMAN, E. E. SPROUL and A. B. GUTMAN, Am. J. Cancer **28**:485, 1936.

The King and Armstrong method for determining the phosphatase activity of serum and bile has been applied to the estimation of the phosphatase activity of bone. The use of a method depending on the estimation of the phenol liberated was found to be advantageous in determining the phosphatase activity of unfiltered aqueous extracts of bone. Values for the phosphatase activity of normal bone from adult human subjects are recorded. The lumbar vertebrae, pelves and ribs exhibit appreciable phosphatase activity; the cranium and the femoral cortex often possess too little phosphatase for quantitative determination. Markedly increased phosphatase activity was noted in bone from the sites of osteoplastic skeletal metastases in a patient with carcinoma of the prostate gland. Sections of bone revealed very active formation of new bone. The phosphatase activity of bone from the sites of osteolytic skeletal metastases secondary to carcinoma of the breast was within normal limits. In this case sections of bone from involved areas showed virtually no reactive proliferation of bone. Examination of a cranium which showed Paget's disease in an advanced stage revealed increased activity of bone phosphatase. It is pointed out that the marked increase in activity of bone phosphatase at the sites of osteoplastic metastases explains the rise in serum phosphatase activity previously noted in a number of patients with widespread osteoplastic involvement of the skeleton. It is suggested that increased production of bone phosphatase at the sites of osteoplastic skeletal metastases makes the local formation of new bone possible or inevitable. The intrinsic capacity of certain tumor cells to stimulate production of adequate amounts of bone phosphatase appears to determine in part the osteoplastic tendencies of the metastases to which the tumor cells give rise. In addition to increased "alkaline" phosphatase activity (p_H 9.1), markedly increased "acid" phosphatase activity (p_H 5.0) of bone was noted at the sites of osteoplastic metastases secondary to carcinoma of the prostate. Metastatic tumor cells arising from carcinoma of the prostate gland apparently retain their capacity to elaborate the "acid" phosphatase found by Kutscher and Wolbergs in normal prostate tissue. It remains to be determined whether in metastatic cells from carcinoma of the prostate the presence of "acid" phosphatase in such concentration is related to the production of "alkaline" phosphatase and to the osteoplastic character of most metastases of prostatic carcinoma.

FROM THE AUTHORS' SUMMARY.

TESTICULAR TUMORS ASSOCIATED WITH MAMMARY, PROSTATIC AND OTHER CHANGES IN CRYPTORCHID DOGS. W. W. GREULICH and T. H. BURFORD, Am. J. Cancer **28**:496, 1936.

Three cases of cryptorchidism in dogs are described. In each animal an undescended testis had become transformed into a tumor, and this alteration was associated with changes in the mammary glands and in the prostate gland somewhat similar to those which may be evoked experimentally by the injection of theelin. Two of the animals were sexually attractive to male dogs, and when from one of them the tumor was removed surgically the animal lost this attractiveness. No information on this point was available in the case of the third dog. Biologic assays of the tumor of the first dog for gonadotropic, and of the tumor of the second animal for estrogenic, hormones were negative. These negative results are not to be considered as conclusively establishing the absence of the

substances which the procedures used purported to assay, for the failure to demonstrate such hormones may have been due to inadequacies in the extraction methods employed. All three animals showed sparsity of hair and increased pigmentation of the abdominal skin. The extent of these integumentary changes in each dog seemed to be roughly proportional to the severity of the changes observed in the adrenal glands. FROM THE AUTHORS' SUMMARY.

MONOCYTIC LEUKEMIA WITH MYELOID HYPERPLASIA AND LOCALIZED TUMOR FORMATION. A. C. P. CAMPBELL, J. L. HENDERSON and J. H. CROOM, J. Path. & Bact. **42**:617, 1936.

Two cases of leukemia are recorded which showed hematologically and histologically a mixed monocytic-myeloid reaction. The nature of the process and its bearing on theories of leukocyte formation are discussed. It is suggested that the process consists of a stem cell hyperplasia with simultaneous differentiation into two distinct cell types. The occurrence in one case of localized tumor formation in the appendix and cecum is noted. FROM THE AUTHORS' SUMMARY.

CARCINOGENIC ACTION OF RADIUM IN THE RABBIT. J. M. ROSS, J. Path. & Bact. **43**:267, 1936.

Radium-filled platinum tubes 1 cm. in over-all length, with filtration through 0.55 mm. of platinum and a content of 0.1 mg. of radium element, were implanted in the tissues of nine rabbits. In six malignant new growths developed in immediate relation to the tubes. The histologic diagnoses of the tumors in relation to the positions of the tubes are as follows: osteogenic sarcoma (1 case), tube in periosteum; spindle cell sarcoma (2 cases), tube in connective tissue; sarcoma, myxomatous (1 case), tube in subcutaneous tissue; squamous cell carcinoma (2 cases), tube in dermis. It is concluded that gamma radiation of weak intensity but prolonged duration is the carcinogenic factor in these cases and that the type of growth produced depends on the nature of the tissue in which the tube is embedded. Epithelial and connective tissues seem equally affected.

FROM THE AUTHOR'S SUMMARY.

SEROLOGIC SPECIFICITY OF TUMOR TISSUE. G. D'ALESSANDRO and L. GAGLIO, Ztschr. f. Immunitätsforsch. u. exper. Therap. **87**:137, 1936.

An ether extract of a Jensen rat sarcoma reacted with a rabbit antiserum produced with watery suspensions of Jensen sarcoma, while ether extracts of the other organs did not react. By proper control experiments, it was demonstrated that the phenomenon was not due to a tumor specificity of the ether extract but to a disturbance of the lipoid structure in tumor tissue. Inoculations of a suspension of a human uterine cancer into a rabbit resulted in formation of a highly tumor-specific antiserum. An analysis of that serum with special adsorption procedures confirmed its tumor-specific nature by demonstrating that the group, the organ and the species specificity were not significant factors.

I. DAVIDSOHN.

IMMUNOLOGIC REACTIVITY OF TUMOR TISSUE FROM MICE. PAULA WILKE, Ztschr. f. Immunitätsforsch. u. exper. Therap. **87**:252, 1936.

Immune serums produced in rabbits with suspensions of tumor tissue from mice have species-specific and heterophilic antibodies. The former reacted with extracts of organs of the rat; the latter, with extracts of horse kidney. A tumor-specific property in the immune serum was indicated by some of the experimental findings, but the results were not conclusive.

I. DAVIDSOHN.

Relations Between Human Cancer and Tuberculosis. G. Heddaeus, Ztschr. f. Krebsforsch. **42**:140, 1935.

Heddaeus regards the several reciprocal relations between cancer and tuberculosis to be of three classes: (1) that in which latent tuberculosis is reactivated by loss of resistance due to the progress of cancer; (2) that in which a local tuberculous lesion serves as a focus for the genesis of cancer, and (3) that of accidental coincidence of tuberculosis and cancer, in which each disease develops independently until one or the other has progressed sufficiently to affect the course of the other. The varying interreactions Haddaeus explains as due to the direct effects of the two diseases plus secondary changes due to their effect on the reticulo-endothelial system. The latter, he believes, will in large part determine which disease will predominate. If sufficiently active, the reticulo-endothelial system may restrain the growth of the cancer. This defensive effect may be stimulated by the tuberculous infection, or, in more advanced cases of tuberculosis, it may be inhibited.

H. E. Eggers.

Primary Carcinoma of the Appendix. D. Blumenkopf, Ztschr. f. Krebsforsch. **43**:182, 1935.

Blumenkopf believes that in the great majority of the reported cases of primary appendical carcinoma the lesions were actually carcinoids, with morphologic findings similar to those of cancer but without the characteristic clinical behavior. He here reports a case which he regards as one of carcinoma on purely morphologic grounds, in which complete cure followed operative excision. The morphologic features on which he makes this diagnosis are not convincingly shown in his photomicrographs.

H. E. Eggers.

Medicolegal Pathology

The Use of Blood Tests in Cases of Disputed Paternity in the Courts of Ohio. H. S. Hyman and L. H. Snyder, Ohio State Law J. **2**:203, 1936.

The authors present the results of blood grouping and M-N tests performed in cases of disputed paternity in Ohio. In two of ten cases definite decisions were possible. In one case, a father of two grown daughters sought a divorce from his wife on the ground that a child recently born to her was not his. The M-N test failed to exclude the husband but the A-B tests proved that his suspicions were correct. In the other case, a widow accused her late husband's brother of the paternity of a child born to her five years after her husband's death. By means of the tests the falsity of her accusation was demonstrated.

The article also includes copies of the New York and Wisconsin laws dealing with the use of blood grouping in cases of disputed paternity, and model laws are outlined for adoption by the other states.

A. S. Wiener.

The Value of Blood Group Determinations. W. Lewinski, Deutsche Ztschr. f. d. ges. gerichtl. Med. **27**:194, 1936.

Lewinski describes his experiences in determining the groups of blood stains in 122 medicolegal cases. In 17 cases the stains were found to be not of human origin by means of the precipitin test. Of the remaining 105 cases, the results were not decisive in 93 because (1) the defendant and the murdered person both belonged to the same group, (2) the unstained portions of clothing gave the same specific reactions as the stain itself, or (3) the stain gave no specific reaction on account of deterioration. Hence in only 12 of the 122 cases were the results of any positive value, and even in these cases they acted only as aids, and not as conclusive evidence.

Lewinski points out the following hindrances to the application of blood grouping to stains of blood or secretions in criminology: (1) The fact that the blood stain is of the same group as the victim's blood is of no direct evidential value since the only definite statement that might be made is that the stain does not come from a certain person. (2) The defendant's claim that the blood stain is not his could not be refuted if he belongs to the same group as the victim. (3) Since the agglutinins deteriorate relatively rapidly, it is usually difficult to identify group O as a positive property. (4) Little is known concerning the conditions or agents which may destroy group-specific substances. (5) Group-specific substances are also found in secretions and excretions (sweat, sputum, urine), and these stain clothing and interfere with the tests. (6) Various animals have group-specific substances resembling human properties A and B.

A. S. WIENER.

ELECTRIC AND HEAT MARKS. S. KOEPPEN and H. GERSTNER, Virchows Arch. f. path. Anat. **295**:679, 1935.

HISTOCHEMICAL DETECTION OF METALS IN CURRENT MARKS. H. GERSTNER, ibid. **295**:691, 1935.

Schridde held that the mark of an electric current is due to heat generated by the passage of the current through the epidermis. Jellinek insisted that the mark is a specific electric effect and warrants the diagnosis of electric injury. Koeppen and Gerstner present a description of the gross and microscopic appearances of accidental marks made by electric current and of lesions caused experimentally by heat. For the latter, guinea-pigs and human skin freshly removed at surgical operation were used. Heat of the proper degree applied by means of a metal wire causes a painless lesion of the epidermis, not to be distinguished grossly or microscopically from the mark made by electric current. Gerstner insists on the histologic identity of marks made by electric current and marks made by heat. In the former there occurs an electrolytic deposition of metal (iron), which can be detected histochemically. Up to the present there has been found no characteristic that is morphologically specific for the action of the electric current in living tissues.

O. T. SCHULTZ.

Society Transactions

PATHOLOGICAL SOCIETY OF PHILADELPHIA

March 11, 1937

ESMOND R. LONG, *President*

HERBERT L. RATCLIFFE, *Secretary*

TUBERCULOUS VEGETATIVE ENDOCARDITIS SUPERIMPOSED ON ACTIVE CHRONIC RHEUMATIC HEART DISEASE. HUGH G. GRADY.

The coincidence of active rheumatic and tuberculous infections, while not rare, is certainly uncommon. Tuberculous endocarditis likewise is an unusual finding, although Baker recently reported seven cases that he had observed and reviewed the literature on the subject. The case to be presented is of especial interest because it was one of tuberculous vegetative endocarditis engrafted on preexisting rheumatic valvulitis in a state of active recrudescence. The lesion was undoubtedly terminal and associated with rapid general miliary spread of the tuberculous infection.

L. W., a Negress aged 22, was admitted to the Philadelphia General Hospital, in the service of Dr. J. D. McLean, April 22, 1936, complaining of cough and a cold. She stated that five years previously she had rheumatism. For the past eighteen months she had attended the cardiac clinic, where a diagnosis of rheumatic heart disease with mitral and aortic valvulitis was made. She had complained of dyspnea, palpitation and edema of the ankle, although at the time of admission she showed satisfactory compensation. Her past medical history was irrelevant, except for frequent sore throat. A cold developed on April 1, 1936, with cough (at first nonproductive), fatigue, anorexia and loss of weight. There was no dyspnea. Examination on admission revealed slight cardiac enlargement, with systolic and diastolic murmurs at the apex transmitted to the axilla. The cardiac rhythm was regular, and the blood pressure was 122 systolic and 90 diastolic. The lungs showed extensive tuberculous involvement of the right upper lobe; this was confirmed by the x-ray picture. The sputum contained large numbers of tubercle bacilli. The Kahn reaction was negative.

Artificial pneumothorax was instituted, but the patient left the hospital against advice in May. She was readmitted on September 10 with similar complaints and in addition had progressive dyspnea and edema of the ankle. Physical examination revealed marked extension of the tuberculous process, which was now bilateral. The cardiac findings were essentially similar to those noted in April. She failed rapidly and died October 1.

At autopsy, eighteen hours post mortem, extensive bilateral fibrocaseous tuberculosis of the lungs was combined with miliary tubercles in the spleen and liver and tuberculous ulceration of the lower part of the ileum and colon. The pericardial cavity contained 250 cc. of pale straw-colored fluid. The heart weighed 250 Gm.; the transverse diameter was 14.5 cm. in situ. The glistening dark red-brown myocardium was slightly flabby. The aortic cusp of the mitral valve was moderately thickened, and the chordae tendineae were definitely shortened and thickened. Along the line of closure of the mitral valve was a row of pinhead-sized red-gray vegetations, slightly friable and firmly attached to the underlying valve. All the other valves appeared normal.

Sections taken from the left ventricular wall and interventricular septum showed, in addition to degenerative changes in the fibers, the coronary vessels surrounded by frayed collagen mats and a few cellular perivascular Aschoff nodules. In sections of the mitral valve were numerous small blood vessels and large numbers of fibroblasts arranged in sweeping rows and columns beneath the endocardial surface. Firmly attached to the valve was a small vegetation composed

of necrotic cellular débris, a few lymphocytes and larger mononuclear cells, the border being formed by fresh deposits of fibrin; the vegetation was definitely not rheumatic. Ziehl-Neelsen staining demonstrated sparsely distributed rod-shaped acid-fast bacilli not only along the surface but well within the vegetation. No organisms were noted in the valve proper.

THROMBOSIS OF A PATENT DUCTUS ARTERIOSUS ASSOCIATED WITH SEPTICEMIA DUE TO STREPTOCOCCUS VIRIDANS. JOSEPH L. FINN.

M. G., a white woman, aged 23 years, was admitted to the Jefferson Hospital, in the medical service of Dr. H. A. Reimann, Oct. 7, 1936. At 2 years of age she had scarlet fever but recovered completely. At 4 years of age she was a patient at the Jefferson Hospital because of dyspnea and was found to have a "machinery hum" in the pulmonic area. Other cardiac sounds were normal, and a diagnosis of congenital heart disease was made. She improved after a rest in bed and was discharged. After this she was seen from time to time in the outpatient department of the hospital.

For several years prior to 1936 she was a marathon dancer, dancing three or four weeks at a time, despite advice against strenuous exercise. In April 1936 she gave birth to a full-term baby. This was her first pregnancy. The delivery was spontaneous and the puerperium normal. At this time the murmur in the pulmonic area was noted again, but symptoms of heart disease were absent.

Four months after delivery she became ill, having fever, night-sweats, weakness, cough and expectoration. She was confined to bed, but her condition grew worse and in a few days was complicated by sharp sticking pains in the left side of the chest. These were exaggerated on deep breathing. When admitted to the hospital, her temperature was 102 F., and she was slightly cyanotic but not dyspneic. Râles were heard in the left scapular region. The apex beat of the heart was slightly displaced to the left, and in the pulmonic area a to and fro murmur was heard, accompanied at times by a slight thrill. Other heart sounds were normal. She was believed to have a congenital lesion of the heart and possibly tuberculosis. However, further study of the lungs ruled out tuberculosis. Her leukocyte count varied between 11,000 and 23,000. Two blood cultures were sterile. The urine was normal, as were the Wassermann and Kahn tests.

During the first two weeks of the patient's stay in the hospital her condition changed little, although the temperature curve, which was of the septic type, was maintained. After this time she began to have increasingly frequent attacks of sharp pain in the chest. These were accompanied by signs of consolidation in small areas in the lungs and by gradual failure in the patient's condition. Sputum was almost absent, and puncture of the lung for bacteriologic study yielded essentially negative findings. On November 11, a third blood culture was taken and Streptococcus viridans isolated. On December 28, severe pain was felt in the right side of the chest. Dyspnea, cyanosis and chilling of the extremities accompanied this and persisted for about one and a half hours, disappearing gradually. During a second attack, two days later, the patient suddenly died.

At autopsy the heart weighed 508 Gm.; all the cavities were dilated, but the valves were normal. The ductus arteriosus was patent but partially occluded by a soft, friable thrombus, which extended into the lumen of the pulmonary artery and adhered to its wall. Both lungs contained many infarcts, but other organs were essentially normal.

ETIOLOGY OF PLASMA CELL MASTITIS. HELEN INGLEBY.

Study of a case of plasma cell mastitis led to a comparison of cases of "chronic lactation mastitis," "infected galactocele," "traumatic fat necrosis" and "plasma cell mastitis." In the sections examined the lesions were sometimes diffuse, sometimes localized, but all showed similar characters. These were: colostrum cells in the epithelium, more or less necrosis of tissue and an exudate which in many places showed plasma cells, eosinophils and giant cells. Irregular development of breast lobules was also a feature. Blood eosinophilia was present in some of the cases.

Other authors have failed to find bacteria in plasma cell mastitis, and my associates and I found none in the first case studied. The conditions enumerated in the foregoing paragraph should have a common cause since the reactions in all are similar and differ in many respects from the usual type of inflammatory reaction. In all the cases studied, certain areas gave a picture resembling that seen in involution following lactation. Milk-like substances are secreted under certain conditions in the breasts of nonpregnant as well as of pregnant women. The splitting of these substances by enzymes might, theoretically at any rate, produce the special type of lesion which all these conditions have in common. Similar reactions were then produced experimentally by the injection of peptonized milk into the breasts of rabbits.

INFLUENCE OF ESTROGENIC SUBSTANCES IN THE STIMULATION OF ABNORMAL GROWTHS. W. U. GARDNER.

Mice from six different strains in which the incidence of spontaneous mammary tumors in multiparous females varied from approximately 0 to 80 per cent have been given varying amounts of one or more pure estrogenic chemicals. In from 15 to 100 per cent of the male mice in different groups from three strains which received large amounts of estrogen over extended periods tumors developed. The type and extent of the mammary development were more or less abnormal, the ducts being cystic and nodular, or diffuse overgrowths frequently occurring. Male mice of three strains in which the incidence of tumors was low tolerated the injections poorly, and no tumors developed. Male mice receiving small amounts of estrogenic substances rarely showed mammary tumors, although their mammary glands underwent extensive proliferation similar to that occurring normally in virgin females. Mammary tumors have been observed in mice receiving theelin, theelol, equilin benzoate (3-benzoate-17-keto-1, 3, 5, 7-estratriene), hydroxyestrin benzoate (3-benzoate-17-hydroxy-1, 3, 5-estratriene) and keto-estrin benzoate (3-benzoate-17-keto-1, 3, 5-estratriene). Mammary tumors have occurred in higher percentages in those groups of mice that were receiving the latter two estrogenic substances. As these preparations have been given in greater amounts (in terms of mouse units), it cannot be said that they are more likely to induce abnormal growths.

In seven mice sarcomas have developed at the sites of injection of the estrogenic chemicals. Three thymic tumors and five uterine tumors have occurred in the animals given injections. In another mouse leukemia developed. After extensive periods of treatment with large amounts of estrogenic substances, the interstitial cells of the testes became enlarged and increased in number, largely replacing the damaged seminiferous tubules. The basophilic and acidophilic cells of the pituitaries of the treated mice underwent degranulation. Though slight hypertrophy of the pituitaries of male mice usually occurs, tumors have not been observed.

April 8, 1937

ESMOND R. LONG, *President*

HERBERT L. RATCLIFFE, *Secretary*

THE ANNUAL CONVERSATIONAL LECTURE: RELATION OF CERTAIN CHEMICAL SUBSTANCES TO THE PRODUCTION OF CIRRHOSIS. WILLIAM C. VON GLAHN, New York.

Experiments to produce cirrhosis with alcohol, phosphorus, copper, tar and arsenic are reviewed. Attention is directed to the fact that in many of these experiments there was a lack of adequate control animals, that often insufficient data are given, so that it is impossible to repeat the experiments exactly, that frequently diets are not stated and that in many of the experiments too few animals were used. Rabbits can be used for this work if a piece of liver is removed for histologic study before the experiment is begun.

The results of experiments done in collaboration with Dr. Frederick Flinn and Dr. W. Franklin Keim Jr. are reported, in which copper arsenate, lead arsenate or sodium arsenate was fed to rabbits. From each of the animals except one a piece of liver was removed for histologic examination before the experiment was undertaken. In most of the experiments the dose of the substance given was so regulated that the animal received from 1.4 to 1.5 mg. of arsenic daily. When cabbage was a part of the diet, it was found necessary to tear the leaves from each other, wash them in water, place them in 1 per cent hydrochloric acid solution and wash again in water to be certain that all arsenate had been removed. Cabbage often has been treated with lead arsenate, and in one analysis 4.32 mg. of arsenic per kilogram was found. Numerous analyses were made for the arsenic contents of the livers of the animals used in the experiments. Cirrhosis was produced most frequently in those animals given one of the arsenates and fed on a diet of hay and oats. As early as three days after ingestion of the arsenate there were found sharply defined groups of necrotic liver cells, situated most often in the vicinity of portal areas. Sometimes the necrotic cells surrounded the portal areas; in other instances they extended from one portal area to another, and somewhat less frequently, from portal area to efferent vein. Less often they were present in the midzonal portion of the lobule or near the efferent vein. Intact bile ducts at times projected into the mass of necrotic cells. Frequently pigment-containing phagocytes clustered about the margin of the mass of necrotic cells. As the necrotic liver cells were removed the stroma collapsed, an ingrowth of connective tissue ensued, and bile ducts proliferated. Of fourteen rabbits fed the diet of hay and oats and given copper arsenate, twelve had cirrhosis; of fifteen given sodium arsenate, fourteen presented cirrhosis; in each of sixteen that received lead arsenate, cirrhosis was found. Thus, of these forty-five animals, in only three was the liver unaffected by the arsenate. Pigment was increased in the liver in thirty of the forty-five animals, and there was jaundice in nine. The capsular surface of the liver was finely granular or coarsely pebbled in twenty-seven, and in many animals irregularity of lobulation or definite increase of connective tissue could be seen on the cut surface. Many of the animals survived more than two hundred days. When carrots and cabbage were added on alternate days to the diet of hay and oats, the incidence of cirrhosis was reduced. Thus, of seventeen animals, four did not acquire cirrhosis.

The most striking result of diet in protecting the liver from the effect of the arsenates was found when only white bread and potatoes were fed. There were twenty-six animals in this group; in only three was cirrhosis produced. The dose of arsenic was approximately a third of that given in the other two experiments, yet the largest amounts of arsenic were found in the livers of this group of animals. Among the control animals, none of the lesions described was found.

Only negative results were obtained in a series of rats and ferrets.

The following conclusions were drawn: Cirrhosis is produced in rabbits given copper, lead or sodium arsenate. Diet is important in protecting the animal.

CHICAGO PATHOLOGICAL SOCIETY

Regular Monthly Meeting, May 10, 1937

CARL W. APFELBACH, *President*

EDWIN F. HIRSCH, *Secretary*

CERTAIN FEATURES OF RETROGRESSIVE CHANGES OF TUBERCLES. HENRY C. SWEANY, ALMA E. TILLOTSON and VINCENT KOZIELEK.

Third dimension studies were made on ossified tubercles to determine, if possible, the mechanism of retrogression. The changes noted could be classed under two headings: degenerative and regenerative retrogression. The former had to do with necrosis, autolysis and calcification within the capsule, and the latter,

with removal of these products and replacement by tissues. The regenerative changes concerned us most. In all the cases studied, resorption resulted from penetration of the capsule by capillaries, and this seems to be the regular order of the changes. These changes were followed by ramification of the capillaries into the center of the tubercle with subsequent formation of lymphoid tissue or marrow, and in about half the lesions, with bone formation. The bone seemed to originate on supersaturation of the fibroblasts with calcium, which proceeded until tissues formed that looked and behaved like bone.

DISCUSSION

S. R. ROSENTHAL: When avian tubercle bacilli enter the tissues of the mammalian lung, there is no necrosis; the lesion produced regresses, and there is complete restitution of the tissues without a scar. Is the human type of tubercle bacillus responsible, or is the necrosis of tissue an important factor in the production of the lesion?

HENRY C. SWEANY: The tubercle is not due to the products of bacillary disintegration alone. The added factor is necrosis of tissue as a result of the action of bacterial toxins. The caseous center of a tubercle presents the combined effect of the inflammatory response rather than simply bacillary substances.

INCIDENCE OF EXTRAPULMONARY PRIMARY TUBERCULOSIS. HENRY C. SWEANY and WILLIAM L. M. MARTINSEN.

In 132 autopsies all calcified foci observed were investigated in order to determine the percental involvement of each portal of entry in this community. Of primary infections 73.4 per cent involved only the lungs and the lymph nodes at the hilus; 79 per cent involved the lungs if those with multiple portals of entry are added; 8.3 per cent involved the gastro-intestinal tract alone; 12.9 per cent involved the gastro-intestinal tract if those with multiple portals of entry are included; 1.8 per cent involved the cervical or head regions alone; 4.5 per cent involved these regions if those with multiple portals of entry are included; 2.2 per cent (three cases) occurred in the pleura; 2.2 per cent (three cases) were "cryptic" in the liver or lymph nodes without a local lesion. In 2.2 per cent (three cases) the organ involved was in doubt. In 5.3 per cent of the autopsies (seven) no calcifications were found. Calcifications of primary foci of hematogenous infection in the liver and spleen were noted in 30.6 per cent of these postmortem examinations.

DISCUSSION

PAUL R. CANNON: Is there true formation of bone in the spleen?

S. R. ROSENTHAL: You have described multiple primary infections involving several viscera. Did you find more than one primary focus in any organ, such as the lung?

HENRY C. SWEANY: True bone was not found in the spleen. Multiple primary foci have been found in many lungs.

MEGAKARYOBLASTIC MYELOMA WITH CRYSTALLINE PROTEIN IN THE RENAL TUBULES. F. D. GUNN and ARTHUR E. MAHLE.

Present interest in multiple myeloma is centered largely in the origin of the tumor cells, the origin and nature of the Bence-Jones protein that is commonly excreted in the urine, and the effect of such excretion on the kidney itself.

A woman aged 57 years, who had the typical symptoms and signs of the disease, with the characteristic roentgenologic picture of the skeleton, and excreted large quantities of Bence-Jones protein, died about five years after the first signs of the disease. The observations at necropsy and at microscopic examination were unusual in that the tumors consisted of pleomorphic cells, of which many were giant forms with complex nuclei, and in that metastases had occurred in the kidney and crystalline protein was present in the tubules of the kidney. The cells did not show the oxydase reaction and resembled in all their tinctorial properties the undifferentiated cells of the bone marrow, the hemocytoblasts and megakaryoblasts. The larger cells contained indented, multiple or lobulated nuclei with prominent

nucleoli. Their cytoplasm was relatively abundant and free from stainable granules except that a few giant cells contained extremely fine azurophil granules. There was no phagocytic activity. In the kidney there was healed mild glomerulonephritis and outstanding nephrosis, in which an accumulation of large quantities of protein in the convoluted tubules, loops of Henle and collecting ducts was a prominent feature. Besides eosinophilic granular substance, cellular detritus and cylindric hyaline masses there were numerous rhomboid crystals and a few rod-shaped and needle-shaped crystals in the dilated, degenerated tubules. At least some of this substance was thought to be Bence-Jones protein, but its exact chemical nature has not yet been determined.

DISCUSSION

P. A. DELANEY: What was the condition of the blood platelets in the circulating blood? Was the clotting of the blood delayed?

F. D. GUNN: The number of platelets in the blood was not estimated, but there was no tendency to bleed. The clotting time was normal. I should not expect an increase of the platelets, because, according to Wright's theory, only mature megakaryocytes would be concerned. The cells observed were immature forms.

CORRELATION BETWEEN THE PATHOLOGIC CHANGES AND ISOLATIONS OF THE VIRUS IN THE 1936 EPIDEMIC OF POLIOMYELITIS IN CHICAGO. PAUL H. HARMON and VICTOR LEVINE.

Postmortem examinations were made of nine patients with poliomyelitis who died at the Municipal Contagious Disease Hospital, Chicago, during the 1936 epidemic. Fresh pieces of spinal cord and brain were taken for inoculation into monkeys, and adjacent portions were examined microscopically. The ages of the patients were from 4 to 30 years. The periods of disease varied from two to fourteen days; the paralyses lasted from one to eight days. When the cases are divided according to the type of onset, there are three in which the condition was fulminating, four in which it was acute and two in which it was subacute. All but one of the patients had bulbar symptoms. One patient was a Negress. From six to twelve places in each central nervous system were examined microscopically. Every level of the cord and brain stem studied had pathologic changes. The most common lesion was the perivascular exudate, usually composed of equal numbers of lymphocytes and leukocytes. Next most common was the interstitial infiltration, showing usually about equal numbers of round cells and leukocytes. Hemorrhages were few; those present were "ring" hemorrhages. Parenchymatous lesions varied from slight swelling of the ganglion cells or neuronophagia to complete disintegration and replacement of the parenchyma. When the parenchymatous lesions were marked, the perivascular exudates were less noticeable. In many instances, the parenchymatous changes were slight, which may explain why with one exception the microscopic lesions were more widespread than the clinical symptoms. The olfactory bulbs from each brain were sectioned at five different levels, and from five to seven sections at each level were examined. In seven instances no pathologic changes were found. In each of two, a single perivascular exudate was discovered in one bulb, at the most proximal level. In these observations we agree with other recent investigators, especially Sabin and Olitsky, and Smith, who think that the virus reaches the brain by some route other than the olfactory tracts.

The material taken for animal experimentation was preserved in 50 per cent glycerin, and 1 Gm. portions were crushed with sand in saline solution, filtered and injected intracerebrally into monkeys (Macaca mulatta). When suspicious symptoms or fever occurred, the animals were killed, microscopic studies were made, and transmission of the disease to monkeys was attempted. Successful inoculations occurred with material from one or more levels in only four of the nine cases, altogether six. One virus from a case in which the disease was fulminant passed through more than one monkey. This virus has so far been passed through six animals. These animals presented various paralyses; none died spontaneously.

An attempt was made to recover the virus from the nasal and rectal washings of six convalescent patients in the 1935, and fourteen more during the 1936, epidemic. Five first passages and two second passages were obtained with virus from the rectal washings of four patients. No virus was isolated from nasopharyngeal washings. These results, without changes in the tissues of the olfactory bulb, suggest that the gastro-intestinal tract may be a portal of entry.

STUDIES IN ENDEMIOLOGY (PERIODICITY IN SUN SPOTS AND IN MORTALITY). WILLIAM F. PETERSEN.

In studies dealing with the effect of the weather (meteorological environment) on disease, it was observed that correlations exist between periods of greater atmospheric turbulence (sun spot cycles) and periods of increased mortality in different states, in the United States as a whole and in Europe. The following years revealed an increase in sun spot activity: 1857-1860, 1868-1870, 1879-1883, 1890-1893, 1902-1905, 1914-1917, 1924-1928, 1934 now approaching a maximum.

The tuberculosis death rate for Chicago revealed periods of increase that paralleled these periods, with maximal numbers of deaths in 1858, 1870, 1881, 1893, 1906, 1915 and 1928.

There have been five major epidemics of meningitis. These reached their crests in 1872, 1881, 1893, 1917 and 1929 and all originated with periods of increase in sun spot activity.

Deaths from puerperal sepsis increased in the same fashion, that is, in 1872-1873, 1881-1883, 1894, 1907-1911 and 1929; only the sun spot period of 1915 was missed. When the number of deaths from puerperal sepsis increased in Chicago it increased at the same time in adjacent states and in Canada. Of greater significance, it increased then in England, Norway and Sweden and in South America, Australia and New Zealand (mortality statistics of the United States Department of Commerce, Bureau of Census). Here one deals with world-wide trends. While possibly due to a change in bacterial incidence, it is much more likely that the change occurs in the population living in the regions of greatest atmospheric turbulence.

When the death rate for the United States registration area is examined, the periods of increase in rate are found associated with periods of increase in rate in Great Britain, and the trends observed in the rate in Great Britain are also evident in the rate in Prussia. That the effect is on man rather than on infecting organisms becomes evident in a study of deaths from exophthalmic goiter or deaths from gastric ulcer. Here the death rate increased not only in Illinois and Michigan at the same time but also in Massachusetts, Vermont and New Hampshire, and similar trends can be observed in Sweden and Great Britain. The same trend was demonstrated for erysipelas, septicemia, appendicitis, pancreatitis, disorders of the gallbladder and other diseases.

The studies reveal a close integration between man and the changes in the atmospheric environment in which he lives.

BUFFALO PATHOLOGICAL SOCIETY

May 29, 1937

KORNEL TERPLAN, *President*

W. F. JACOBS, *Secretary*

CAUSES OF DEATH IN NEW-BORN INFANTS. MARGARET WARWICK.

The observations at autopsy on 500 new-born infants were analyzed for the chief causes of death. Among the infants 91, or 18 per cent, were dead before birth, as shown by maceration. Trauma occurred in 121, or 24 per cent; in this group, cerebral hemorrhage was present in 91, or 18 per cent, massive hemorrhage

into the adrenals in 17, or 3 per cent, evidence of craniotomy in 6 and rupture of the liver or spleen in 7. Of the total number of infants, 88, or 18 per cent, showed improper aeration of the lungs, due to partial or complete atelectasis in 28, or 6 per cent, to aspiration of amniotic fluid in 24, or 5 per cent, and to pneumonia in 24, or 5 per cent; 60, or 12 per cent, showed malformations incompatible with life, such as craniorachischisis, which occurred in 23, or 5 per cent, and fatal cardiac anomalies, which were observed in 8, or 2 per cent; 11 infants died of diseases peculiar to the new-born (melena neonatorum, erythroblastosis and congenital syphilis), and 7 died of intercurrent infection (meningitis and peritonitis). Only 20, or 4 per cent, showed no demonstrable pathologic lesions.

DEMONSTRATION OF BACTERIAL HEMOLYSIN IN EXPERIMENTAL AND NATURAL INFECTIONS. ERWIN NETER and EDMUND UHRY JR.

In a recent report on the production of streptococcic fibrinolysin in vivo, it was mentioned that hemolysin may be found in exudates of mice infected with hemolytic streptococci (Neter, E.: *Proc. Soc. Exper. Biol. & Med.* **34**:735, 1936). The experiments now presented are concerned with a more detailed study of the production of hemolysin by various bacteria in experimental and natural infections.

The following technic was employed: The bacteria, suspended in sterile salt solution, were injected intraperitoneally into mice; from twenty-four to forty-eight hours later the peritoneal cavity was washed with about 10 cc. of salt solution. The supernatant fluid was tested for hemolytic activity. Smears of the peritoneal exudate and cultures of the cardiac blood were also made. For the demonstration of the hemolytic activity the supernatant fluid of the peritoneal washing was serially diluted and mixed with equal amounts of a 1 per cent suspension of red blood cells.

Of twelve strains of hemolytic streptococci obtained from human lesions, eleven produced hemolysin when injected into mice; the twelfth strain failed to show production of hemolysin in vivo in spite of the fact that it was pathogenic for mice and produced hemolysin in 10 per cent serum broth. The titer of the hemolysin in such peritoneal washings may be as high as 1:100, and higher after two hours' incubation. With the fluid in stronger concentration, hemolysis may occur within from ten to fifteen minutes. The peritoneal washings from these mice were lytic for the red corpuscles of sheep, rabbits, guinea-pigs, mice and man, although the susceptibility of the cells was not uniform. The hemolytic activity of the peritoneal washings sometimes disappeared when the specimens were incubated at 37 C. for twenty-four hours, while the specimens kept in the ice chest still showed hemolytic potency. Heating of the exudate at 55 C. for five minutes resulted in a marked loss in hemolytic activity. Peptone markedly accelerated the hemolytic activity of the peritoneal washings, an effect which is similar to the known influence of peptone on the hemolysin obtained in vitro.

Two strains of Staphylococcus aureus-hemolyticus, which killed mice in from twenty-four to forty-eight hours and which could be recovered in pure culture from the cardiac blood, also produced hemolysin in vivo. Of four strains of hemolytic Bacillus coli, two were found to be pathogenic for mice. When injected intraperitoneally, they were recovered in pure culture from blood from the heart. These two strains were injected into ten mice. The peritoneal washings of eight of these showed hemolytic activity.

To determine whether bacterial hemolysin is also produced in infections of man, various exudates were tested for hemolytic activity. Four of six exudates from patients with empyema due to Streptococcus haemolyticus proved hemolytic; exudates from three patients with peritonitis, one with pericarditis and one with purulent arthritis— all of these conditions being due to Str. haemolyticus—showed hemolytic activity. Some of the specimens were filtered through a Seitz filter shortly after being obtained; the filtrate showed hemolytic activity, although to a markedly less degree than the original fluid.

EPENDYMAL CYST OF THE CERVICODORSAL REGION OF THE SPINAL CORD. IRVING HYMAN, WALLACE B. HAMBY and S. SANES.

A case of an ependymal cyst in the cervicodorsal region of the spinal cord in a 7 year old boy was presented. No similar instance was found reported in the available literature.

The patient complained of pain in the cervical region for five months. The pain was intermittent and radiated down the left side of the neck. The left arm was somewhat cold and cyanotic. The neck muscles were rigid; there was pain on motion of the neck, as well as signs of a disturbance of the pyramidal tracts. X-ray films revealed flattening of the pedicles of the cervical vertebrae and incomplete fusion of the laminae of the seventh cervical vertebra. On spinal puncture, a block was demonstrated, and a lesion was definitely localized at D-1 by injection of iodized poppy-seed oil 40 per cent into the cisterna magna. The preoperative diagnosis was that a congenital tumor was present in the cervicodorsal region of the spinal cord, with skeletal anomalies in the cervical vertebrae. At operation a cyst was found anterior to the spinal cord and attached to the cord, chiefly at the level of the first dorsal vertebra. A portion of the cyst was removed. The cyst fluid was clear. The excised specimen was a part of the wall of the cyst. The lining, which was well preserved, consisted chiefly of columnar cells with basal nuclei and slightly blue-staining borders. In some areas the cells possessed cilia; in other sites the cells were cuboid. The wall was composed of collagenous connective tissue. In the inner part, the fibrous tissue was loose, the capillaries showed injection and there were recent hemorrhages and slight signs of chronic nonspecific inflammation. In the outer part, the fibrous tissue was compact. An explanation for the occurrence of the cyst was offered in a local evagination of the floor plate of the neural tube, with subsequent isolation and cyst formation.

MARKED MACROCYTIC ANEMIA IN "HEMOLYTIC JAUNDICE" WITH FINDINGS IN THE STERNAL BONE MARROW AND SPLEEN. NORMAN HEILBRUN.

An 18 year old school girl complained of tiredness for four months. She also suffered from anorexia and a dull ache in the left upper quadrant of the abdomen. Ten days before admission to the hospital she had nosebleed. She was pale. There was puffiness of the face. The temperature was 103.5 F. The conjunctivae were slightly icteric. A soft systolic murmur was heard over the entire precordium. The spleen was definitely palpable. The hemoglobin was 24 per cent (Sahli), and there were 1,120,000 erythrocytes per cubic millimeter. Because of the extreme illness of the patient, a blood transfusion of 500 cc. of citrated blood was given immediately after admission.

On the next day examination of the blood showed:

Hemoglobin	6.2 Gm. per 100 cc.
Erythrocytes	1,550,000
Leukocytes	4,500
Polymorphonuclears	
Segmented	42%
Nonsegmented	5%
Monocytes	2%
Lymphocytes	51%
Normoblasts	4 per 100 white cells
Megaloblasts	1 per 100 white cells
Hematocrit reading	18.7%
Reticulocytes	2-5%
Mean corpuscular volume	120 cubic microns
Mean corpuscular hemoglobin	40 micromicrograms
Mean corpuscular hemoglobin concentration	33%

The blood film, treated with Wright stain, showed definite macrocytosis of the red cells. Polychromatophilia and stippling were fairly prominent. No abnormal white cells were present. The number of platelets appeared to be moderately reduced. The fragility test showed that hemolysis began at 0.50 per cent and was complete at 0.36 per cent. The serum bilirubin was 2.4 mg. per hundred cubic

centimeters and gave a delayed van den Bergh reaction. Free hydrochloric acid was present in the gastric content. The urine was normal except for the presence of urobilinogen in a dilution of 1:40. There was no blood in the stools.

Marrow taken from the sternum at this time was soft, deep red and extremely cellular. In a smear stained by the Giemsa method there was marked predominance of red cells, with a large number of primitive erythrogonias and many megaloblasts. Nevertheless, the tendency toward normoblastic maturity was present, as there were many young normoblasts. The section showed extreme cellularity. No fat was present. There were nucleated red cells in various stages of maturity, as noted in the smear.

Because of the marked macrocytosis, and even though there was evidence that blood was being destroyed, large doses of liver extract were given intramuscularly, and the patient made satisfactory improvement in the course of from three to four weeks. The reticulocytes rose to about 15 per cent, and the red cell count showed a rise to 3,500,000, with hemoglobin 70 per cent. The serum bilirubin dropped to 1.5 mg. per hundred cubic centimeters. The fragility test remained the same, and the reticulocytes still averaged 5 or 6 per cent. The spleen did not change in size at any time.

On account of the continued destruction of blood and lack of reduction in size of the spleen, splenectomy was done. The spleen weighed 755 Gm. Microscopically, the markings were well preserved; the reticulum was distended. The meshes of the reticulum were filled with blood, and the sinusoids were compressed. The appearances were typical of those in hemolytic jaundice. The patient made an uneventful recovery; the level of the blood bilirubin dropped to normal, and the hemoglobin and red cells rose to normal. Two weeks after splenectomy the fragility test remained as previously reported. There was no history of jaundice in the family.

This case is reported because of the unusual marked macrocytic anemia during a relapse rather than the usual microcytic hyperchromic anemia with spheroid cells.

Book Reviews

The Patient and the Weather. By William F. Petersen, M.D., and Margaret E. Milliken, S.M. Volume 1, Part 2: Autonomic Integration. Cloth. Pp. 781, with 366 illustrations. Price, $9. Ann Arbor, Mich.: Edwards Brothers, Inc., 1936.

This voluminous number in Petersen's series of monographs was preceded in publication by volumes 2 and 3 and part 1 of volume 1. This order of publication required inclusion in preceding volumes of fundamental material that is covered more fully in this volume.

The present volume deals "primarily with normal autonomic integrations." The author states his objective thus: "In the development of the monographs of this series I have a simple objective, fundamentally clinical. That is to make obvious the close integration of the human organism and the immediate atmospheric environment." In carrying out that objective, so far as this volume is concerned, the author discusses "constitution . . . Then . . . the limits of biological reaction as regulated by the autonomic apparatus; the meteorological environment . . .; finally, studies of this integration as made evident in the observations of normal individuals living in the midst of the storm tracks."

The first 149 pages are devoted to the individual human being and his atmospheric environment. This is followed by the main portion of the volume, in which are set forth, by means of a study of "the three major components of the autonomic apparatus—chemical, endocrine, nervous," the reactions of the normal person to his atmospheric environment. The material includes observations on nineteen adults of various types and of both sexes and on four children. The study of the first four adults represents a prodigious amount of labor. The data are presented in tables and graphs and are analyzed and discussed. The various graphs employed exhibit a high degree of ingenuity. Each of the comprehensive graphs, in the form of an insert, exhibits not only ingenuity in preparation but a surface area of 400 square inches (2,580 sq. cm.) and a complexity greater than that of an automobile road map and a railroad time-table combined. "In order to facilitate a rapid survey, the chemical data have been graphically portrayed, because tabulation of figures would be even more fatiguing than an examination of the charts, intricate though they may seem on inspection."

Chapters 10, 11 and 12 present the results of further studies on changes in the blood and on variability in serologic reactions. Chapter 13 deals with measurements of somatic activity, especially of psychopathic persons. Chapter 14 returns to the air and discusses season, climate and climatic cycles. The final chapter summarizes the author's views under the title "The Human Organism as a Cosmic Resonator."

In preceding volumes medical educators, clinicians and pathologists received a mild scolding for merely talking about the weather but doing nothing about it. In the present volume it is the turn of the physiologists and editors. "Actually one searches the literature in vain for detailed observations that might have some bearing on the subject of biologic rhythm and meteorological association. The physiologists have shown little interest." "For the moment it is not the mode to discuss the meteorological environment; consequently, no proper medical editor will countenance papers treating the meteorological relations to disease." Speaking of one of his co-workers, the author says, "Due credit should be given him despite the fact that, apart from a single brief note, it has not been possible to obtain separate publication for his studies." Speaking of certain observations on the excretion of creatinine he makes this statement: "These, being unorthodox, could not be accepted for publication in the proper journals." One wonders whether these words were written in a mood engendered by the COD phase of increasing sourness or acidity brought about by a polar infall.

Petersen's presentation, in word and in graph, is convincing, and the evidence has been piled high. But after one puts aside the volume and gets out in an atmosphere ionized by summer sunshine, one is not so sure. It is true that the biologic and meteorological graphs reveal the same general trends, but peaks and valleys do not always coincide. Is this the result of negation? Do meteorological state and biologic reaction necessarily have a causal relationship? In dealing with so many variables on both sides of the equation, in atmospheric environment and in human reactions, is it possible that a synchronism that is interpreted as cause and effect may be a matter of coincidence and chance? Is it possible that atmospheric changes and biologic reactions may vary independently of each other but in the same general direction as the result of some perhaps extraterrestrial force? The final section of the next to last chapter of Petersen's volume deals with sun-spots.

The Avitaminoses. The Chemical, Clinical and Pathological Aspects of the Vitamin Deficiency Diseases. By Walter H. Eddy, Ph.D., Professor of Physiological Chemistry, Teachers College, Columbia University, and Director of Bureau of Foods and Sanitation, *Good Housekeeping Magazine*; and Gilbert Dalldorf, M.D., Pathologist to the Grasslands and Northern Westchester Hospitals, Westchester County, New York. Cloth. Price, $4.50. Pp. 338, with 29 illustrations. Baltimore: Williams & Wilkins Company, 1937.

According to the preface, this book is an expansion of the "Vitamin Manual" by the senior author, which was published some fifteen years ago. As indicated in the title, it deals with the chemical, clinical and pathologic aspects of the diseases due to vitamin deficiencies. In his foreword James Ewing points out that the book is the first in English to set forth fully the value and the results of the application of morphologic methods to the study of vitamins. "The enormous literature on vitamins has been collated and digested and reliable conclusions presented in condensed and accessible form for the general physician and pathologist. It will now be possible to determine more accurately how frequently deficiency diseases occur among the sick in general and hospital practice. A reliable clue is also available to the general pathologist in the interpretation of various morbid conditions, metaplasia, disorders of collagen formation, degenerative changes in the nervous system, etc." This statement well describes the book, which consequently will be of direct interest to pathologists. The nature and functions of each vitamin are discussed, and the clinical and anatomic manifestations of deficiency are described. The morbid changes in structure caused by lack of vitamins are well illustrated. There are chapters on standard methods for vitamin bio-assay, on clinical tests and on vitamin values of foods. The style is clear. There are some mistakes in spelling, e. g., "Carlsson" for Carlson, "Wahlgreen" for Wahlgren, "Kupfer" for Kupffer, "cervitamic" for cevitamic. One wonders why *Gerüstmark* should be used. But there can be no question of the value of the book as a source of information concerning avitaminosis.

Books Received

MÉCANISME DES RÉACTIONS FERMENTAIRES: SON ÉTUDE SUR L'AMYLASE ET L'INVERTINE. L. Ambard, professeur à la Faculté de Médecine de Strasbourg, and Mlle. S. Trautmann, assistante de Laboratoire à la Faculté de Médecine de Strasbourg. Paper. Price, 35 francs. Pp. 104. Paris: Masson & Cie, 1937.

OXYGÉNOTHÉRAPIE ET CARBOTHÉRAPIE: BASES PHYSIOLOGIQUES. APPLICATIONS CLINIQUES. TECHNIQUES. L. Dautrebande, professeur à la Faculté de Médecine de l'Université de Liége. Paper. Price, 55 francs. Pp. 300. Paris: Masson & Cie, 1937.

THE DEVELOPMENT OF CARDIAC ENLARGEMENT IN DISEASE OF THE HEART: A RADIOLOGICAL STUDY. J. H. Palmer. Medical Research Council Special Report Series, no. 222. Paper. Price, 1 shilling. Pp. 49, with 58 figures. London: His Majesty's Stationery Office, 1937.

PRÉCIS DE MÉDECINE COLONIALE. C. Joyeux, professeur de parasitologie a la Faculté de Médecine de Marseille et ex-médecin colonial, and A. Sicé, professeur a l'Ecole d'application du corps de Sante colonial de Marseille. Second edition. Cloth. Price, 170 francs. Pp. 1250, with 240 illustrations. Paris: Masson & Cie, 1937.

THE HARVEY LECTURES. Delivered Under the Auspices of the Harvey Society of New York, 1936-1937, Under the Patronage of the New York Academy of Medicine. Dr. Wilder Penfield, Dr. Eugene M. Landis, Dr. S. Walter Ranson, Dr. R. Schoenheimer, Dr. Thorvald Madsen, Dr. Herbert S. Gasser, Dr. C. N. H. Long, Sir Henry Dale. Series XXXI. Cloth. Price, $4. Pp. 245, with illustrations. Baltimore: Williams & Wilkins Company, 1937.

CLINICAL PARASITOLOGY. Charles Franklin Craig, M.D., M.A. (Hon.), F.A.C.S., F.A.C.P., Col., U. S. Army (Retired), D.S.M., Professor of Tropical Medicine, Tulane University of Louisiana, New Orleans, and Ernest Carroll Faust, M.A., Ph.D., Professor of Parasitology in the Department of Tropical Medicine, Tulane University of Louisiana, New Orleans. Cloth. Price, $8.50. Pp. 733, with 243 illustrations. Philadelphia: Lea & Febiger, 1937.